Sandoz
Atlas of Haematology



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Preface

The rapid advances in haematology which have taken place during the last few years have been matched by the publication of so many new text-books and atlases that the literature has been kept well up-to-date. Despite the excellence of the existing works, however, it seemed to us that there was still room for another atlas of haematology designed to meet the needs of the practising physician. Nowadays, haematology is no longer the hobby of a few specialists, but has become an indispensable diagnostic aid to the modern physician. It is important, therefore, that he should possess an atlas which will enable him to identify without difficulty the corpuscles of the blood and haemopoietic organs. The coloured drawings usually employed almost inevitably suffer from the disadvantages of subjective interpretation. Our aim has been to produce an atlas in which the blood cells are faithfully portrayed and the appearance is that actually seen when a well prepared film is examined under the microscope. This has been accomplished by taking colour photomicrographs of the various preparations, by using modern techniques of colour printing and by avoiding artificial combinations of cells from different parts of films. In this way, we have been able to achieve an authentic reproduction of the form and colour of the blood cells in their natural surroundings.

The reception accorded to the first edition of the "Sandoz Atlas of Haematology", which was published in 1949 in French and German only, was extremely favourable and completely justified the undertaking. In a short time, the entire edition was exhausted, and the continued demand for the atlas made it necessary to prepare a second edition. It was decided that this new edition should appear not only in French and German but also in English and Italian, and that the atlas should be revised and enlarged. This has been done and a further 34 illustrations, consisting of 70 separate photographs, have been added.

With a few exceptions, a magnification of 1 : 1200 has been employed. The magnification of 1 : 500, usual in microscopic work, although employed for actually taking the colour photographs proved too low for purposes of reproduction. In order to obtain the necessary clarity and the same wealth of detail seen in a transparent slide, a higher magnification is needed for reproductions to be examined under incident light. In the case of a few particularly large elements, such as megakaryocytes, stroma cells and osteoclasts, however, a lower magnification was used in certain photographs in order to show the cells in their entirety.

The atlas is divided into three sections, each of which follows the same general plan. The first part of each section deals with the development of the nine species of blood cells in the haemopoietic tissues, particularly in the bone marrow, and this is followed by a description or

photographs of the cells in the peripheral blood under normal and pathological conditions. As no international agreement on the question of terminology has yet been reached, the classical nomenclature has been employed as far as possible.

Part I gives a brief account of the basic principles of haematology and of the technique of preparing and staining blood and bone marrow films. At the end of this section, will be found drafts of forms suitable for recording the results of blood and bone marrow counts, followed by the normal values of haematological data.

Part II is devoted to a systematic description of the various groups and species of blood cells and their development.

Part III contains 44 plates comprising a total of 256 illustrations of normal and pathological elements found in the blood and haemopoietic organs. Apart from Figures 78 and 79 C, which show leucocytes in rabbits with Pelger-Huet's anomaly, and Figures 248 B and 248 C showing trypanosomes in guinea pig blood, all the photographs are of human blood cells. Facing each plate will be found descriptions of the individual photographs.

Since the atlas is intended primarily to fill practical needs, every effort has been made to include all known cells of the blood and haemopoietic organs, whether normal or pathological, for the general practitioner is just as likely as the specialist to encounter the rarer elements.

Many cells, for example tissue basophils and certain stem cells, are only rarely seen in healthy persons, but under pathological conditions their numbers may be increased without qualitative changes, and even in leukaemic patients, the blood corpuscles may be normal in appearance. It was therefore possible to overcome the difficulty of obtaining photographs of some of the rarer elements by using preparations from pathological cases.

Of the 579 photographs which go to make up the 256 illustrations, 346 are of preparations in our own collection, while the preparations used for the remaining 233 photographs were kindly placed at our disposal by clinicians in Switzerland and other countries We should like to record here our gratitude to the undermentioned for the help they have given us in this connection (individual acknowledgments will be found in the descriptive text facing the illustration).

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Swiss Tropical Institute, Basle· Prof. R. Geigy, Dr. Kathe Schäffer.

Children's Hospital, Basle: Prof. E. Freudenberg, Dr Margrit Esser, Dr. F Hauser.

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Dr. W. Baumgartner, Interlaken, Switzerland; Dr. Nellie Haverkamp Begemann, Leyden, Holland; Prof L. Berman, Detroit; Prof. P. Chevallier and Dr G. Marinone, Paris; Dr. H. Goldeck, Hamburg; Dr. F. Heckner, Göttingen; Prof. L. Heilmeyer and Dr. H. Begemann, Freiburg, Germany; Prof. C. Jiménez-Diaz, Dr. G. Paniagua and Dr. J. Perianes, Madrid; Prof. L. R. Limarzi, Chicago; Dr. P. Lopes Cardozo, Leyden; Dr. J. Mallarmé, Paris; Prof. C Monge M. and Prof. P. Weiss, Lima; Prof. H. Nachtsheim, Berlin; Prof. A. Owren, Oslo; Dr. A. Perret-Gentil, Basle; Dr. A. Piney, London; Dr. K. A Punschel, Arosa, Switzerland; Dr. M. Pio da Silva, São Paulo, Brazil; Dr B. Steinmann, Bern; Dr. B Wiedemann, Olomouc, Czechoslovakia.

We are especially indebted to Dr. Käthe Schäffer who selected the specimens containing blood parasites and arranged the photographs on plates 42 and 43.

Our appreciation and thanks are also due to Messrs. Frobenius Ltd , Basle, for their skill and willing co-operation in the difficult and laborious task of reproducing the colour photographs.

The "Sandoz Atlas of Haematology" has been written and compiled by Dr. E. Undritz of the Sandoz Pharmacological Research Laboratories, under the direction of Prof. E. Rothlin.

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PART ONE

General Considerations

I. Fundamental Principles of Haematology

This general section is devoted to a brief discussion of the terminology and classification of the blood corpuscles, followed by a description of their formation, reproduction, maturation, functions, and destruction (lysis), both under normal and under abnormal conditions. An account is also given of the cellular elements which are found in the bone marrow but are not concerned in haemopoiesis. Detailed descriptions of the individual blood cells will be found in Part Two While the atlas accurately reflects the present state of our knowledge of haematology, no claim can be made to completeness, for there are many questions still to be answered

1. TERMINOLOGY

Haematology possesses no uniform terminology, partly because there are still gaps in our knowledge and partly because not all authors interpret the findings in the same way. In the following pages, we shall adhere to the classical nomenclature, both for the two large *groups* of blood corpuscles—the erythrocytes and the leucocytes—and for the individual cell species: normocytes and megalocytes, basophils, eosinophils, neutrophils, monocytes, lymphocytes, plasma cells and the elements of the megakaryocyte-platelet system. Each of the individual cell species undergoes various stages of development to which numerous synonyms have been applied. We have selected those names which appear to agree best with the specific properties of the elements they describe and which are least likely to be misunderstood For the ripe neutrophils, for example, the term "polynuclear" is incorrect, since they possess only one nucleus On the other hand, the description "segmented" is accurate, though it naturally refers only to the nucleus and not to the entire cell. Similarly, we have preferred the older term "myelogenous" to the now more generally adopted term "myeloid", since the latter strictly speaking means "resembling marrow" and not "derived from marrow". By employing only classical terms and those derived from them, we hope to have arrived at an internationally acceptable nomenclature and, as far as possible, we have used the same terms in all four languages. Terms which differ from these but are in common use in certain countries have also been added in brackets to facilitate understanding. For cell stages which have only recently been recognized, we have used terms which fit in well with the classical terminology, such as

Table 1

The Genealogical Table of the Blood Cells[1]

A. Primary Formation in the Embryo[2]

Fertilized ovum → mesoderm → mesenchyme → specific stem cells

B. Somatic Regeneration[3]

Normally found in	Proliferation (reproduction)	Species of Blood Corpuscles: Normocytes	(Megalocytes[4])	Megakaryocyte-Platelet system	Basophils	Eosinophils	Neutrophils	Monocytes	Lymphocytes	Plasma Cells
Haemopoietic Organs	Proliferation	**Pronormoblast** → Macronormoblast	**(Promegaloblast)**	**Megakaryoblast**	**Basophiloblast**	**Eosinophiloblast**	**Neutrophiloblast**	**Monoblast**	**Lymphoblast**	**Plasmoblast**
		↓ Basophilic normoblast (also from Macronormoblast)	↓ (Basophilic megaloblast)	↓	↓ Basophilic promyelocyte	↓ Eosinophilic promyelocyte I ↓ Eosinophilic promyelocyte II	↓ Neutrophilic promyelocyte I ↓ Neutrophilic promyelocyte II	↓	↓	↓
		↓ Polychromatic normoblast ↓ Oxyphilic normoblast I	↓ (Polychromatic megaloblast) ↓ (Oxyphilic megaloblast I)	Promega-karyocyte	↓ Basophilic myelocyte	↓ Semi-mature eosin. myelocyte ↓ Mature eosin myelocyte	↓ Semi-mature neutr myelocyte ↓ Mature neutr. myelocyte ↓ Neutrophilic metamyelocyte	Promonocyte	Prolymphocyte	Proplasmocyte
	Limit of Proliferation									
	No Proliferation	↓ Oxyphilic normoblast II	↓ (Oxyphilic megaloblast II)	↓ Megakaryocyte	↓ Basophilic metamyelocyte	↓ Eosinophilic metamyelocyte ↓ Staff eosinophil	Juvenile neutrophil ↓ Staff neutrophil	↓ **Monocyte**	↓ **Lymphocyte**	↓ **Plasmocyte**
	Entrance into blood stream	↓	↓	↓ **Blood platelets**	↓ **Segmented basophil**	↓ **Segmented eosinophil**	↓ **Segmented neutrophil**			
Blood		Pronormocyte Reticulocyte ↓ **Normocyte**	(Promegalocyte Reticulocyte) **(Megalocyte)**	Destruction						

[1] See text on p 3 [2] Commencing with the fertilized ovum [3] Commencing with the specific stem cells [4] Cells in brackets are not normally found in adults

stem cells and the definitive forms are printed in heavy type

basophiloblast, eosinophiloblast and neutrophiloblast, to describe the ungranulated stem cells of the corresponding species of blood corpuscle. Naegeli's "myeloblast" and Ferrata's "haemocytoblast" are now known to be collective terms covering a variety of stem cells. Since the introduction of bone marrow puncture, it has also proved necessary to subdivide many of the more mature stages of development. Thus the promyelocytes of the basophils, eosinophils and neutrophils are now subdivided into promyelocytes I and promyelocytes II. The promyelocytes I are the same size as the blast cells but already contain sparse granulation (corresponding to Ferrata's basophilic, eosinophilic and neutrophilic myeloblasts), while the larger cells with more abundant granulation are known as promyelocytes II (the promyelocytes of Naegeli or the immature myelocytes of Rohr). These terms also fit in well with the classical nomenclature and are not likely to lead to misunderstanding. Instead of the misleading term "reticulocyte" we speak of "proerythrocyte", which conforms better to the classical terminology. Certain terms, such as "staff form", although not classical have found general acceptance, and these have been retained. Others, such as "Türk's irritation cell", have been dropped, since it is no longer certain to which cells they were originally intended to apply. To facilitate understanding, however, the most widely employed synonyms have also been given.

2. CLASSIFICATION OF THE BLOOD CELLS INTO GROUPS, SPECIES AND STAGES OF DEVELOPMENT

Table 1 shows the genealogical table of the blood cells. Two distinct processes are shown: (a) the *primary formation* of the specific stem cells in the embryo, starting from the fertilized ovum, and (b) the *somatic regeneration* of the different species from their stem cells, which sets in after the latter have been elaborated in the embryo.

The following phases in the process of development are indicated: the limit at which proliferation ceases (broken line), the threshold at which the various blood corpuscles enter the blood stream (thin, continuous line) and the stage of development at which the death and destruction of the cells eventually occurs (thick, continuous line). Stages which correspond to approximately the same degree of maturity are shown at the same level. It will be seen that the stage of development at which entrance into the blood stream and death of the cell occurs varies according to the species of blood corpuscle to which it belongs. The plasma cells and lymphocytes do not reach the same stage of maturity as, for example, the neutrophils, while the destruction of the latter takes place at an earlier stage than that of the normocytes.

With regard to the nomenclature of the red blood corpuscles, confusion can be avoided if the two species, normocytes and megalocytes, are referred to collectively as erythrocytes. The introduction of new terms is thus rendered unnecessary. By employing the prefix "normo-" systematically for all the stages of development of the one series, from pronormoblast to normocyte, and the prefix "megalo-" for the other series, from promegaloblast to megalocyte, a clear differentiation between the two series can be made (see Table 1). The prefix "erythro-" should only be employed when a differentiation is unnecessary, and conveys no more than "red blood corpuscle".

Table 2

CLASSIFICATION OF THE BLOOD CORPUSCLES INTO GROUPS AND SPECIES

Groups	Species or systems	Synonyms
Erythrocytes	Normocytes	(Erythrocytes in a restricted sense, rubricytes)
	Megalocytes	
Leucocytes	Basophils with soluble granulation	Blood basophils (blood mast cells)
	Eosinophils	(Acidophils)
	Neutrophils	(Microphages)
	Monocytes	(Large mononuclear and intermediate forms, macrophages; vaguely related to histiocytes, migratory cells, clasmatocytes, reticulo-endothelial and endothelial cells, etc., see p 58)
	Lymphocytes	(Small mononuclear forms)
	Plasma cells	Plasmocytes (plasmacytes, Türk's irritation cells [?])
	Megakaryocytes Blood platelets	Giant cells of the bone marrow Platelets (thrombocytes)

Terms in brackets are those not employed elsewhere in the atlas

Table 2 shows how the blood corpuscles are divided into two *groups*, the erythrocytes and the leucocytes, which are then subdivided into *9 species (polyphyletism* according to Undritz, 1934). The most common synonyms are also given.

Classification of the species into stages of development*

A. THE SYSTEM OF THE NORMOCYTES (Plates 2 and 6, and parts of Plates 1, 3, 4 and 5): Pronormoblast, macronormoblast (macroblast)**, basophilic normoblast, polychromatic normoblast, oxyphilic normoblast with nuclear structure, oxyphilic normoblast with structureless (disintegrating) nucleus, *pronormocyte* (normocytic reticulocyte, normocyte with vital granulation, granulofilocyte), *normocyte*

B THE SYSTEM OF THE MEGALOCYTES (Plate 7 and parts of Plates 1, 3, 4 and 5): Promegaloblast, basophilic megaloblast, polychromatic megaloblast, oxyphilic megaloblast with

* The stages which normally pass into the blood stream from the haemopoietic centres are printed in italics

** The macronormoblast or macroblast is an intermediate stage which does not invariably occur.

nuclear structure, oxyphilic megaloblast with structureless (disintegrating) nucleus, promegalocyte (megalocytic reticulocyte), megalocyte.

C. THE SYSTEM OF THE BASOPHILS WITH SOLUBLE GRANULATION (*Blood basophils*, Plate 11): Basophiloblast (myeloblast of the basophilic series), basophilic promyelocyte stage I (basophilic myeloblast of Ferrata), basophilic promyelocyte stage II, basophilic myelocyte, basophilic metamyelocyte, *segmented basophil*.

D. THE SYSTEM OF THE EOSINOPHILS (Plates 12 and 13): Eosinophiloblast (myeloblast of the eosinophilic series), eosinophilic promyelocyte stage I (eosinophilic myeloblast of Ferrata), eosinophilic promyelocyte stage II, eosinophilic myelocyte, eosinophilic metamyelocyte, staff eosinophil, *segmented eosinophil*.

E. THE SYSTEM OF THE NEUTROPHILS (Plates 14—19): Neutrophiloblast (myeloblast of the neutrophilic series), neutrophilic promyelocyte stage I (neutrophilic myeloblast of Ferrata), neutrophilic promyelocyte stage II (immature myelocyte), semi-mature neutrophilic myelocyte, mature neutrophilic myelocyte, neutrophilic metamyelocyte, juvenile neutrophil, *staff neutrophil, segmented neutrophil* (Metamyelocytes and juvenile forms are usually grouped together and described simply as "metamyelocytes" or simply as "juvenile forms". Arneth subdivides the segmented forms into those with 2, 3, 4, 5 and more segments).

F. THE SYSTEM OF THE MONOCYTES (Plates 20—23): Monoblast, promonocyte, *monocyte*.

G. THE SYSTEM OF THE LYMPHOCYTES (Plates 24 and 25): Lymphoblast, *prolymphocyte, lymphocyte*.

H. THE SYSTEM OF THE PLASMA CELLS (Plates 26—28): Plasmoblast, *proplasmocyte, plasmocyte*.

I. THE SYSTEM OF THE MEGAKARYOCYTES AND PLATELETS (Plates 29—31): Megakaryoblast, promegakaryocyte, megakaryocyte, *blood platelet*.

The precise characteristics of the individual groups, species and stages of development of the blood cells are described in Part Two.

3 ORIGIN OF THE BLOOD CELLS

Certain cells of the adult organism, such as the nerve and muscle cells, no longer possess the power of proliferation and hence, under normal conditions, they cannot be replaced. In the case of other cells, such as those of the skin and the glands, however, there is a steady formation of new cells to take the place of those constantly being used up. The blood cells belong to the class of proliferative cells.

In the embryo, the blood cells are formed in the mesenchyme which is derived from the mesoderm. The sites of formation of the blood cells vary in a definite order during the period of intra-uterine development. At first situated outside the embryo, in the yolk sac and abdominal pedicle, and later within the embryo, chiefly in the liver and spleen, they finally become localized in the bone marrow and lymphatic tissue. At birth, this process of development is already complete

Despite its name, the lymphatic tissue is not solely concerned with the formation of *lymphocytes*, although lymphopoiesis is strongly evident in it. From the same tissue arise the *monocytes* and the *plasma cells* as well as the *basophils with insoluble granulation* (tissue basophils) although, in man, the latter do not properly speaking belong to the blood cells. Lymphatic tissue is found not only in the lymph glands, the lymph follicles and the spleen, but also in vascular sheaths throughout the body.

The bone marrow is the site of formation of *basophils with soluble granulation* (blood basophils), *eosinophils, neutrophils, megakaryocytes* and *normocytes*. However, *lymphocytes, monocytes, plasma cells* and *basophils with insoluble granulation are also produced in the bone* marrow in the vicinity of the vessels.

In certain primary and secondary diseases of the blood, the blood cells which are normally formed only in the bone marrow can also arise in other organs, particularly in the spleen, the liver and the lymph glands. On the other hand, it may also happen that lymphocytes, monocytes, plasma cells, and basophils with insoluble granulation are formed in excessive quantities in the bone marrow. In man, the megalocyte is unique in that it appears for only a brief period during one particular stage of development. It is found in the embryo from the second week of pregnancy, when blood formation commences, up to the third month of pregnancy, its formation being entirely extra-embryonic. According to Naegeli, megalocytes reappear in adults in pernicious anaemia, and are then formed principally in the bone marrow.

Many authors have assumed that the supply of new cells in the adult organism is derived, as in the embryo, from multipotent mesenchymal cells. It seems more probable that, after the initial embryonic period, each species of blood cell has its own particular stem cell *(polyphyletism)*, for each cell species follows its own independent line of development, beginning with the stem cell and ending with the mature form which is released into the blood. Although there are many points of resemblance among the different cell species with regard to the form, structure and staining reactions of the cells, and of their nuclei and cytoplasm, each individual species of cells exhibits a number of specific characteristics by which it may be distinguished from other species. Typical features are also to be found in the chemical composition of the cells, and in their enzyme content and functions. Above all, it has so far been impossible to provide conclusive proof of the existence either of intermediate forms between one species and another or of mitoses or polyploid bastards of different cell species. There is thus no evidence that cells from different species can be derived from common stem cells.

4. THE BLOOD CORPUSCLES UNDER NORMAL CONDITIONS

a) **Reproduction and maturation.** All blood cells reproduce by mitosis. The number of consecutive mitoses is limited, for when the cells mature they eventually lose their power of division. No longer capable of proliferation, they enter the blood stream, fulfil their functions and are destroyed. In healthy individuals, it is only in extremely rare instances that cells in mitosis are found in the blood, but dividing plasma cells, or perhaps lymphocytes, may very occasionally be seen (Figs. 155 A, 155 B).

It is probable that the new cells necessary for the continual replacement of those which have been destroyed are formed from *generative stem cells*, which divide to give somatic and generative stem cells. The *somatic stem cells* develop by repeated mitosis and maturation until they reach the sterile end stage and are used up. On the other hand, the generative stem cells do not mature, but remain as primitive cells which preserve the pattern for future generations ("Belegzellen"). In certain plants the generative stem cells can be distinguished morphologically from somatic stem cells, but in the case of the blood corpuscles no apparent difference exists

Our understanding of the normal and pathological forms exhibited by the cells of the blood and haemopoietic organs in the course of their development has been greatly increased by the knowledge gained through recent studies in cytology. Of particular importance in this connection is the discovery that polyploid cells, the existence of which in botany and zoology has been recognized for decades, also occur in the haemopoietic organs (see pp. 19—24).

With the exception of the megakaryoblasts, the blood cells remain *diploid* during the process of reproduction, one parent cell giving rise to two separate daughter cells. On the other hand, the megakaryocytes develop by *polyploidy* into giant cells, Figs. 161—166 (see pp 21, 22 and 65).

Division of the blood corpuscles is accompanied by a striking change in the cytoplasm, which ceases to be homogeneous and takes on a curious, mottled appearance (Figs. 32, 34 A, 35 A, 37, 38, 135, 136, 155 and 157). In the case of the erythroblasts, there is also a very sudden increase in the haemoglobin content during mitosis. Consequently, the different stages of development—the basophilic, polychromatic and oxyphilic erythroblasts—are very easy to distinguish from one another. In the case of the other blood cells, there is no such marked connection between maturation and mitosis, for they mature without interruption, even between mitoses and after their last mitosis. Their different stages of maturation cannot therefore be so sharply differentiated, and there is an important subjective element in their interpretation. In the early stages of development, mitoses may take place without visible changes in structure and staining power. The final division of the blood cells occurs in the haemopoietic centres, except, as already mentioned, in the case of certain plasma cells and lymphocytes

During maturation, changes take place in the nucleus and cytoplasm of the blood corpuscles. Morphological, structural, physical and chemical criteria are employed to assess maturity.

In all the very young stages of development, the cell **nucleus** is round. In the case of the erythrocytes (Figs 2 and 4), lymphocytes (Figs. 131 and 132) and plasma cells (Figs. 143—146), it normally remains round, even in the mature cell, and no segmentation occurs. The nucleus of the monocytes is often indented (Figs. 46 F and 51 B) or irregular in shape, even in the monoblast stage, segmentation seldom takes place (Fig 110 F). In the case of the basophils with soluble granulation (Figs. 55—58), as well as in the eosinophils (Figs. 61—63) and the neutrophils (Figs. 71—74), the nucleus not only develops indentations during the later development but also exhibits segmentation. The segments remain connected with one another by fine threads or bridges. For each species of cell, segmentation proceeds according to definite laws The majority of mature eosinophils have nuclei with two typical segments, the neutrophil nuclei have three. In the case of the basophils, no such regularity is found, the nuclei taking on a variety of shapes. The giant nucleus of the megakaryocytes has a bizarre shape (Figs. 166

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Many authors have assumed that the supply of new cells in the adult organism is derived, as in the embryo, from multipotent mesenchymal cells. It seems more probable that, after the initial embryonic period, each species of blood cell has its own particular stem cell *(polyphyletism)*, for each cell species follows its own independent line of development, beginning with the stem cell and ending with the mature form which is released into the blood. Although there are many points of resemblance among the different cell species with regard to the form, structure and staining reactions of the cells, and of their nuclei and cytoplasm, each individual species of cells exhibits a number of specific characteristics by which it may be distinguished from other species. Typical features are also to be found in the chemical composition of the cells, and in their enzyme content and functions. Above all, it has so far been impossible to provide conclusive proof of the existence either of intermediate forms between one species and another or of mitoses or polyploid bastards of different cell species. There is thus no evidence that cells from different species can be derived from common stem cells.

4. THE BLOOD CORPUSCLES UNDER NORMAL CONDITIONS

a) **Reproduction and maturation.** All blood cells reproduce by mitosis. The number of consecutive mitoses is limited, for when the cells mature they eventually lose their power of division. No longer capable of proliferation, they enter the blood stream, fulfil their functions and are destroyed. In healthy individuals, it is only in extremely rare instances that cells in mitosis are found in the blood, but dividing plasma cells, or perhaps lymphocytes, may very occasionally be seen (Figs. 155 A, 155 B).

It is probable that the new cells necessary for the continual replacement of those which have been destroyed are formed from *generative stem cells*, which divide to give somatic and generative stem cells. The *somatic stem cells* develop by repeated mitosis and maturation until they reach the sterile end stage and are used up. On the other hand, the generative stem cells do not mature, but remain as primitive cells which preserve the pattern for future generations ("Belegzellen") In certain plants the generative stem cells can be distinguished morphologically from somatic stem cells, but in the case of the blood corpuscles no apparent difference exists.

Our understanding of the normal and pathological forms exhibited by the cells of the blood and haemopoietic organs in the course of their development has been greatly increased by the knowledge gained through recent studies in cytology. Of particular importance in this connection is the discovery that polyploid cells, the existence of which in botany and zoology has been recognized for decades, also occur in the haemopoietic organs (see pp. 19—24).

With the exception of the megakaryoblasts, the blood cells remain *diploid* during the process of reproduction, one parent cell giving rise to two separate daughter cells On the other hand, the megakaryocytes develop by *polyploidy* into giant cells, Figs. 161—166 (see pp. 21, 22 and 65).

Division of the blood corpuscles is accompanied by a striking change in the cytoplasm, which ceases to be homogeneous and takes on a curious, mottled appearance (Figs. 32, 34 A, 35 A, 37, 38, 135, 136, 155 and 157). In the case of the erythroblasts, there is also a very sudden increase in the haemoglobin content during mitosis. Consequently, the different stages of development—the basophilic, polychromatic and oxyphilic erythroblasts—are very easy to distinguish from one another. In the case of the other blood cells, there is no such marked connection between maturation and mitosis, for they mature without interruption, even between mitoses and after their last mitosis. Their different stages of maturation cannot therefore be so sharply differentiated, and there is an important subjective element in their interpretation. In the early stages of development, mitoses may take place without visible changes in structure and staining power The final division of the blood cells occurs in the haemopoietic centres, except, as already mentioned, in the case of certain plasma cells and lymphocytes.

During maturation, changes take place in the nucleus and cytoplasm of the blood corpuscles. Morphological, structural, physical and chemical criteria are employed to assess maturity.

In all the very young stages of development, the **cell nucleus** is round. In the case of the erythrocytes (Figs. 2 and 4), lymphocytes (Figs. 131 and 132) and plasma cells (Figs. 143—146), it normally remains round, even in the mature cell, and no segmentation occurs. The nucleus of the monocytes is often indented (Figs. 46 F and 51 B) or irregular in shape, even in the monoblast stage; segmentation seldom takes place (Fig 110 F). In the case of the basophils with soluble granulation (Figs 55—58), as well as in the eosinophils (Figs. 61—63) and the neutrophils (Figs. 71—74), the nucleus not only develops indentations during the later development but also exhibits segmentation. The segments remain connected with one another by fine threads or bridges. For each species of cell, segmentation proceeds according to definite laws The majority of mature eosinophils have nuclei with two typical segments, the neutrophil nuclei have three. In the case of the basophils, no such regularity is found, the nuclei taking on a variety of shapes. The giant nucleus of the megakaryocytes has a bizarre shape (Figs. 166

Despite its name, the lymphatic tissue is not solely concerned with the formation of *lymphocytes*, although lymphopoiesis is strongly evident in it. From the same tissue arise the *monocytes* and the *plasma cells* as well as *the basophils with insoluble granulation* (tissue basophils) although, in man, the latter do not properly speaking belong to the blood cells. Lymphatic tissue is found not only in the lymph glands, the lymph follicles and the spleen, but also in vascular sheaths throughout the body.

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and 178 B). As maturation proceeds, the nuclei of the blood corpuscles grow gradually smaller and more compact and their structure becomes denser and coarser. The nuclei of the normoblasts and megaloblasts disperse completely.

In the fine reticular structure of the nuclei of young blood cells, *nucleoli* are present. When not obscured by chromatin, these give a clear blue coloration with panoptic staining. The number and appearance of the nucleoli vary according to the species of cell. Thus, the lymphoblasts (Figs. 131 B and 133 B) usually have only one nucleolus, whereas all other types of cell have two, three or more nucleoli. The nucleoli can be distinguished most clearly in the neutrophiloblasts (Figs. 46 D, 51 A, 88 and 89) where they are relatively large and surrounded by a narrow, compact border of chromatin. In the monoblasts, the nucleoli are poorly visible (Figs. 46 E, 46 F, 51 B, 107, 108 D and 122 A). In the case of the eosinophiloblasts (Figs. 45 B, 45 C and 61 A), the structure of the nucleus is so dense that, in place of nucleoli, only large violet "Hofs" surrounded by condensed chromatin can be distinguished. In the erythroblasts, the nucleoli are large and are recognizable by their clear blue centres, while their borders glisten through the dense chromatin (Figs. 2, 3, 22, 25, 31 and 42). — As the cell grows older, the nucleoli are absorbed. In their place, spherical homogeneous masses of condensed chromatin, the so-called "post-nucleolar chromatin masses", may remain, even in segmented nuclei. The clarity with which the nucleoli are visible depends upon the type of stain employed and on how thinly the cells are spread in the smear. Using the simple Giemsa staining technique and the peroxidase reaction of Graham-Knoll, the nucleoli do not show up as well as with the staining method of Pappenheim and Wright and the modified peroxidase reaction for eosinophils. The more spread out the nucleus is, the more easily visible are the nucleoli (Fig. 90). They are most conspicuous and most readily counted in the so-called "nuclear shadows", the crushed nuclei of damaged cells with round nuclei. Even in mature lymphocytes, the blue nucleolus sometimes becomes visible when they are injured in this way (Fig. 234).

The substance composing the **cytoplasm** may be basophilic, acidophilic (oxyphilic, eosinophilic), or polychromatic. Acid, basophilic cytoplasm stains with basic dyestuffs, basic, acidophilic cytoplasm with acid dyestuffs. Polychromatic cytoplasm has an amphoteric reaction an[illegible] stains with both basic and acid dyestuffs.

All *young* blood cells contain basophilic cytoplasm, which becom[illegible] ...ly oxyphili[illegible] as maturation proceeds. The erythrocytes, blood basophils, eosinoph[illegible] ... and [illegible] karyocytes develop completely oxyphilic cytoplasm by the time they [illegible] On the ot[illegible] hand, the cytoplasm of the monocytes undergoes [illegible] whi[illegible] lymphocy[illegible] and plasma cells remains entirely basophilic. [illegible] of [illegible] basophilic cytoplasm stains *blue* and oxyphili[illegible] (Fig. 4[illegible] leucocytes (basophils, eosinophils and [illegible] [illegible]ig. cytes from lymphatic tissue (lymphocytes, pla[illegible] [illegible] ink Consequently, in the case of mature leucocytes, [illegible] blue colour of the cytoplasm whether they are [illegible] haemopoietic tissue. A knowledge of this e[illegible] blood films, especially to the beginner (see Table 6, [illegible]

Granulation of the cytoplasm can occur in all species of blood cell with the exception of the plasma cells; for many types it is the rule. In the cytoplasm of the more mature leucocytes, true azurophilic, basophilic and eosinophilic granules become visible on panoptic staining.

The azurophilic granules contain acidic substances which take up basic dyes (azur), the original colour of the dyestuff being altered in the process This phenomenon is known as "metachromasia". With the usual stains, the azurophilic granules assume a brilliant, dark purple colour and have the shape of dense clusters of sharply defined splinter-like bodies or crystals (Auer rods or Auer bodies, Figs. 87 and 92). The azurophilic granulation ("progranulation") which appears in the earliest stages of development of the eosinophils (Fig. 61), neutrophils (Fig 71) and monocytes (Figs. 51 B and 108), vanishes again as maturation proceeds. A permanent azurophilic granulation appears at a later stage of development in certain lymphocytes (Figs. 44 D, 132 B, 134 B and 137), and in the megakaryocytes (Figs. 166 and 178) and the blood platelets derived from them (Figs. 173 and 174). The fine azurophilic granulation visible in the later stages of the monocytes (Figs. 109—119) is probably derived from ingested material. As in the case of the nucleoli, the clarity with which the azurophilic granulation is visible depends very much upon the stain employed. It shows up better with Pappenheim stain than with Giemsa

Basophilic granulation is found only in the cytoplasm of the blood and tissue basophils (Figs 43 B, 45 A, 55—60, 191 and 192) The granules contain heparin, the sulphuric acid ester of a nitrogen-containing polysaccharide This substance has a very pronounced acid character and the granules therefore exhibit a much stronger metachromasia than azurophilic granules. They stain reddish violet with panoptic stains and with the "specific" toluidine blue stain (Figs. 57 D and 192 H), even the nuclei of the basophils taking on a pronounced reddish coloration The granules are round and of various sizes In the case of the blood basophils, mere fixation with methyl alcohol is often sufficient to dissolve out the granules (Figs. 43 A and 58) and they therefore show up only weakly or not at all on subsequent treatment with Giemsa stain. For this reason, these cells are often referred to as "basophils with soluble granulation". The granulation of the tissue basophils is not dissolved by methyl alcohol (Fig. 192 G) and they are therefore also known as "basophils with insoluble granulation".

The eosinophilic granules (Figs 61—70) of the eosinophils contain a spermine derivative; they are spherical, approximately uniform in size, and have a vesicular appearance. Owing to their strongly alkaline reaction, they stain intensely with the acid stain eosin. Their formation takes place in what at first appear as small, round, achromatic areas in the eosinophiloblasts (Figs 45 B and 61 A), and they sometimes pass through an intermediate azurophilic stage.

The specific granulations of the blood basophils and eosinophils make their appearance early and persist for the entire life of the cell. Both these species of cell are therefore named according to the nature of their granulation

A granulation is known as "neutrophilic" when it reacts neither acidophilic nor basophilic with triacid staining, although this method is no longer in common use. With the panoptic stains generally in use to-day, the neutrophils occasionally show a fine azurophilic granulation (Fig 74 B), in addition, fine, dense, acidophilic granulation may be seen on intense Pappenheim staining (Figs 43 B, 45 C and 44 A).

Using specific stains, it is possible to detect constituents of the cytoplasm which do not show up with the usual methods. In young, anuclear erythrocytes, or proerythrocytes (reticulocytes), basic stains, such as brilliant cresyl blue, reveal a basophilic substance which has a filamented and nodular appearance and is known as *substantia granulofilamentosa* (Fig. 18).

Summing up, it may be said that maturation ceases at different stages of development in different species of cell. Plasma cells and lymphocytes commence functioning as relatively young cells, which complete their activity and are destroyed without further development. In the case of the monocytes, destruction occurs at a later stage of maturation, while a still later stage is reached by the blood basophils, the eosinophils and the neutrophils. The highest degree of maturity is that attained by the erythrocytes, the entire nucleus of which is finally absorbed In a completely different category is the development of the megakaryocytes. After their peculiar polyploidal growth to giant cells, they discard large shreds of cytoplasm into the blood stream and these separate to form the blood platelets. Thus, in this case, the mature functioning unit is not a cell but merely a fragment of a cell.

b) **Functions.** Once they have reached full maturity and become incapable of further proliferation, the blood cells enter the blood stream and begin fulfilling their functions, either there or, in the case of certain cells, in the tissues into which they subsequently pass. The monocytes begin their activity while still at the site of their formation and the same probably applies also to the plasma cells. The functions of the blood corpuscles remain to a large extent still unknown; even though a particular species of blood cell may be shown to possess a certain function, it is by no means probable that this covers the entire range of its activity. The following facts, however, may be considered established: the *erythrocytes* act as oxygen carriers by reason of their haemoglobin content, the *basophils* produce the anticoagulant substance heparin and the *eosinophils* apparently play a role in allergic disorders, the exact nature of which is not yet clear. From the high enzyme content of the *neutrophils* it may be concluded that they take part in metabolic processes; in the form of so-called "microphages", they assist in the fight against infection by phagocytosis of bacteria. The "monocytes" are also very rich in enzymes Their specific function is the defence and cleansing of the organism by active phagocytosis (Figs. 111—118) and storage (athrocytosis, colloidopexia, Figs. 115—121), thus removing pathogenic micro-organisms, foreign bodies and debris. The function of the *lymphocytes* is still uncertain. The *plasma cells* are held to be responsible for the formation and interconversion of certain plasma proteins, while the *blood platelets* take part in the process of coagulation by providing thrombokinase and by their power of agglutination.

c) **Destruction.** Mature cells which have fulfilled their function are being continuously destroyed and replaced by new ones—*patriam inserviendo consumor.* "Disintegrating cells" (necrobiotic and necrotic cells) are those cells which are themselves in the process of destruction (*Figs.* 131 B and 227—232), whereas "destructive cells" (Figs. 111—118) are those which digest other cells (principally the monocytes or reticuloendothelial cells). Certain organs, including those in which haemopoiesis takes place, are responsible for the destruction of the blood cells. Principally concerned are the spleen, the lungs, the intestine, the lymph glands, the bone

marrow and the liver. Destruction is brought about enzymatically and takes place very rapidly. It is therefore rare to find disintegrating cells in the peripheral blood in healthy persons (Figs. 227 and 228). In all the blood cells, with the exception of the erythroblasts, destruction of the nucleus and the cytoplasm takes place almost simultaneously. In the case of the erythroblasts, destruction proceeds in two stages, disintegration of the nucleus occurring within the cell while it is still in the bone marrow, and the denucleated cytoplasm then passing into the blood stream as a mature, functioning erythrocyte, destruction of which takes place much later.

d) **Artefacts.** By preparing smears of the samples obtained from the blood and the haemopoietic organs, it is possible to spread out the cells more thinly and with less damage than can be achieved when wet fixation or sections are employed. Nevertheless, even in making smears, some cells, particularly those obtained by marrow puncture, are liable to be damaged if they are stretched beyond their elastic limit. It is important to recognize these artefacts and not to mistake them for intact cells. Certain types of artefact are repeatedly encountered, depending upon the nature of the cell and the degree of trauma. With the exception of the lymphocytes, all blood cells in the early stages of development, when the nuclei are still round, possess a certain resistance, in consequence of which they are only flattened by slight trauma and not crushed. The cytoplasm, however, may be squeezed out in the form of projections and the structure of the chromatin and the nucleoli then becomes more clearly visible (Figs. 235 and 236). This gives the appearance of a histioid structure and such cells may be mistaken for young, multipotent cells. If the trauma is more severe, the cytoplasm is crushed and becomes dispersed in the surrounding blood plasma (Fig. 233). The nucleus remains as a blurred smear, the so-called "nuclear shadow". Round nuclear shadows can be produced from all blood cells with round nuclei and not only from lymphocytes. Those obtained from the latter cells constitute the well-known "Gumprecht's shadows" (Fig. 234). The lymphocytes are less resistant than the other cells with round nuclei and are therefore more frequently crushed. The cytoplasm may either become dispersed or appear in the neighbourhood of the nuclear shadow in the form of a spray of fine droplets of the same order of size as the platelets (Fig. 234). This difference in behaviour between damaged lymphocytes and other leucocytes which have been damaged may help to clarify the diagnosis in doubtful cases of stem-cell leukaemia.

The *diameter* of the cell as seen in the blood film may also be artificially increased and may appear twice as great in thin portions of the smear as in thicker ones (Fig. 90). Monocytes appear larger in blood films than mature neutrophils (Fig. 50). In actual fact, both types of cell are the same size, as can be confirmed in thick portions of the smear (Fig. 103 C), in "thick drop" preparations, in sections, and in gold-shadowed smears. The monocytes merely flatten out to a greater extent than the neutrophils in the preparation of films.

5 THE BLOOD CORPUSCLES UNDER ABNORMAL CONDITIONS

The cellular composition of the blood may deviate from the normal both qualitatively and quantitatively under a variety of conditions: (a) *constitutional or inherited anomalies;* (b) *reactive* (secondary or symptomatic) changes due to local or systemic diseases, a consequence of the close relationship between the blood and the organs; (c) *primary* changes in diseases

regarded as primary affections of the haemopoietic parenchyma or of the blood itself. The abnormal conditions are manifest in the form of disturbances in reproduction, maturation, release of the cells into the blood stream and destruction of blood cells.

a) **Hereditary familial anomalies of the blood corpuscles.** These fall into two groups. The *first group* comprises anomalies due to *primary structural peculiarities* which lead to characteristic morphological changes in the blood cells, sometimes manifest only under certain conditions. Seven anomalies of this type are known.

Anomalies of the erythrocytes:

1. *Elliptocytosis* (ovalocytosis, Figs. 7, 8 and 30).
2. *Drepanocytosis* (sickle cell anomaly, Figs. 9 and 117 B).

Anomalies of the leucocytes:

3. *Pelger-Huët's anomaly* (Pelger-Huët's nuclear anomaly of the leucocytes, pseudo-regenerative leucocyte picture) Figs. 77, 97 and 101 A.
4. *Hereditary hypersegmentation of the neutrophils*, Fig. 80.
5. *Hereditary increase in segmentation of the eosinophils*, Fig. 64 B.
6. *Alder's anomaly of leucocyte granulation*, Figs. 181 and 182.
7. *May-Hegglin's polyphylic disturbance of maturation*, Figs. 183 and 184.

Individuals in whom all the cells of the species affected by the anomaly are abnormal, i.e. those exhibiting the heterozygotic or homozygotic manifestation of the anomaly, are known as "complete carriers" (Figs. 7, 77 A, 77 B, 78 A and 78 B). Those in whom only a certain percentage of the cells exhibit the anomaly, the rest being normal, are described as "partial carriers" (Figs. 8, 77 C and 78 C).

Of the above anomalies, Pelger-Huët's anomaly has also been found in rabbits (Fig. 78).

The primary, morphological peculiarities underlying these anomalies of the blood corpuscles are found as normal characteristics in certain species of animals.

The *second group* of anomalies consists of those in which the changes in the blood corpuscles are *secondary* to a systemic affection. Two anomalies of this type are known: *microspherocytosis* in congenital haemolytic jaundice (Fig. 10) and *erythroblastosis* in Cooley's anaemia (thalassanaemia, Mediterranean anaemia, leptocytosis). Microspherocytes are ordinary normocytes which have taken on a more spherical shape as the result of haemolysis. The release of erythroblasts into the blood stream in Cooley's anaemia is likewise secondary in nature, the causes being as yet unknown.

A third anomaly which possibly belongs likewise to this category is the *megalocytosis* occurring in pernicious anaemia (Addisonian anaemia, Biermer's disease, Figs 3, 4, 6, 24, 25 C, 27 and 42). Here there is a marked fall in the production of normocytes (Fig. 27) with a reappearance of the megalocytes normally present only in the early embryonic period. The precipitating cause is lack of the anti-pernicious-anaemia factor. In the cryptogenic form, the cause appears to be of a constitutional, possibly hereditary nature.

b) **Reactive (secondary) changes.** A great variety of causes may give rise to reactive changes in the blood corpuscles. The transition from normal to pathological is a gradual one and the two conditions often overlap. The blood picture is in a state of very labile equilibrium

capable of wide variations within physiological limits. These fluctuations are greatest in infants, less marked in young children and smallest in adults (see Normal values, pp. 37 and 38). Large individual variations are also found. The diagnosis of reactive changes may be a very difficult one—more so, for example, than that of certain hereditary anomalies which can be recognized by a mere glance into the microscope. In reactive changes the morphological findings must always be interpreted against the background of the entire clinical picture.

The distinction between normal and pathological may be particularly difficult when the changes are of a quantitative nature only. Thus, in certain circumstances, a leucocyte count of 12,000 may indicate an infective process necessitating immediate treatment, whereas, in other cases, an increase to as much as 20,000 may occur in a healthy individual as the result of a morning's fast, particularly if the autonomic nervous system is somewhat unstable. A leucocyte count of 3,000 may still be normal, but it can also be evidence of a serious disease, e.g. panmyelophthisis.

Qualitative changes in the haemogram, such as the nuclear shift, often provide much more information than quantitative alterations.

The various species of blood corpuscle may each react differently, or they may respond in groups, or they may all behave similarly. The reactive changes include, in the first place, all those which result from a lack of essential blood-forming substances. Such disorders frequently affect erythropoiesis for which iron is needed. The iron reserve may become exhausted as a result of chronic or periodic haemorrhage, or the absorption of iron may be impaired by gastro-intestinal disturbances. In this way, the various *iron-deficiency anaemias* are produced. These manifest themselves in the form of a diminution in the number of erythrocytes, a reduction in their haemoglobin content and changes in their volume, diameter and appearance (Figs. 15 and 16). Many of the erythrocytes released into the blood stream are immature forms, consisting principally of abnormally large numbers of the anuclear proerythrocytes containing *substantia granulofilamentosa* (Fig. 18). Sometimes normoblasts are also found. When properly administered, in the correct dosage, a suitable iron preparation will cure these forms of anaemia provided they are not due to an incurable condition, such as carcinoma. Infective diseases may also lead to anaemic states with similar alterations in the erythrocytes. In the severe allergic reaction occurring in infants following Rhesus incompatibility (haemolytic disease of the new-born), large numbers of erythroblasts may be discharged into the blood stream (Fig. 22). Erythropoiesis may be paralysed by a number of causes, as for example by hypersplenism or by certain poisons. These give rise to the aplastic form of anaemia, in which there is no deficiency of essential blood-forming substances.

It has already been pointed out that lack of another important substance, the anti-pernicious-anaemia factor, is responsible for the development of Addisonian anaemia. The disease can be cured by administration of the missing factor.

The existence of disturbances in leucopoiesis due to lack of blood-forming substances has not been conclusively proved. The most frequent causes of reactive changes in the leucocytes are infections, intoxications and allergic disorders. *Leucocytosis* means an increase and *leucopenia* a decrease in the number of white blood corpuscles in unit volume of blood. Frequently, only one system reacts, e.g. the neutrophils in acute infections (Fig. 75) or the eosinophils in allergic disorders (Fig. 65). Quantitative changes in the peripheral blood do not necessarily

indicate similar changes in the haemopoietic organs and *vice versa*. The complete or almost complete disappearance of the neutrophils from the circulation is known as *agranulocytosis, granulocytopenia* or, more correctly, as *aneutrophilocytosis*. *Alymphocytosis* is the almost complete disappearance of the lymphocytes from the peripheral circulation. *Thrombopenia* indicates a decrease in the number of blood platelets. *Panhaemocytophthisis* (Glanzmann) or *panmyelophthisis* is the name given to a marked diminution in all types of blood cells, especially the *erythrocytes, neutrophils and megakaryocytes of the bone marrow, accompanied by* corresponding changes in the blood. If the bone marrow is, at the same time, still rich in cells (increased production of lymphocytes, plasma cells, monocytes and tissue basophils), the condition is termed *panmyelopathy*.

c) **"Primary" changes.** An example of a "primary" increase in the *erythrocytes* is *polycythaemia vera* (Vaquez disease, Osler-Vaquez disease), in which the leucocyte count is also increased. This disease should not be confused with the purely symptomatic "polyglobulia" (erythrocytosis, polycythaemia spuria), which is a reactive increase affecting only the erythrocytes.

"Erythraemia" or *acute erythraemic myelosis* (di Guglielmo) is a fatal hyperplasia of the normocytic system.

"Primary" diseases of the *leucocytes* are known as *leukaemias*. They may affect only one species of leucocyte or several species simultaneously. Leukaemias involving only one species may affect any of the leucocytes, and can be classified as follows.

Basophils: Basophilic leukaemia with predominance of
- a) basophiloblasts and basophilic promyelocytes, Fig. 45 A.
- b) mature basophils, Figs. 55 and 59.

Eosinophils: Eosinophilic leukaemia with predominance of eosinophilic myelocytes, Figs. 67 and 68.

Neutrophils: Neutrophilic leukaemia with predominance of
- a) neutrophiloblasts (myeloblasts), Figs. 88 und 100.
- b) neutrophilic promyelocytes I, Fig 90.
- c) neutrophilic promyelocytes II, Figs. 91—94.

Monocytes. Monocytic leukaemia with predominance of
- a) monoblasts, Fig. 123.
- b) promonocytes.
- c) monocytes.

Lymphocytes. Lymphocytic leukaemia (lymphadenosis) with predominance of
- a) lymphoblasts, Figs. 133 B, 141 and 142.
- b) lymphocytes, Figs. 134 and 140.

Plasma cells· Plasma cell leukaemia with predominance of
- a) plasmoblasts and proplasmocytes, Fig 150.
- b) plasmocytes.

Megakaryocytes. Megakaryocytic leukaemia, Fig. 170.

The name *"mixed leukaemias"* is given to those forms in which both myelogenous and lymphatic elements are simultaneously affected Strictly speaking, the common *chronic*

myelogenous leukaemia (chronic myelosis, Figs. 66, 89, 95, 96 and 189) is also a mixed leukaemia, since the blood basophils, the eosinophils, the neutrophils, the megakaryocytes and sometimes the plasma cells are all present in increased numbers. "*Erythroleukaemias*" have been described in which immature leucocytes are released into the blood together with large numbers of normoblasts. The question arises here, whether this flooding of the blood with normoblasts is merely a consequence of their crowding-out by the hyperplastic bone marrow or is due to the secondary anaemia which is present at the same time.

Distinctions are made between *acute* and *chronic* leukaemias and between *leukaemic* and *aleukaemic* leukaemias. Acute leukaemias lead to death within, at the most, a few months, whereas the chronic forms generally last two or three years, and occasionally ten years or longer. In acute leukaemias, hyperplasia is usually confined to a single species of very immature leucocyte. In chronic myelogenous leukaemia, hyperplasia of several species of cell occurs, while in chronic lymphatic leukaemia, only the lymphocytes are affected. In these cases, the cells released into the blood are, for the most part, more mature than in the acute leukaemia. A leukaemia is *leukaemic* in the strict sense of the word when the absolute leucocyte count is in excess of the normal, and it is *aleukaemic* when the count is normal or reduced. If the leucocyte count is alternately increased, normal and reduced, one speaks only of leukaemic or aleukaemic stages or phases. In the spontaneous aleukaemic phase, pathological cells are still present in the blood, if they have disappeared, a true remission has occurred.

The plasma cells are liable to a special type of abnormal proliferation. In contrast to the other species of blood cells, the plasma cells may be considered more as fixed cells, since, under normal conditions, they are always to be found in the tissues in which they are formed, appearing only rarely and in small numbers in the blood. It is probably for this reason that primary overproduction of this species of cell more frequently leads to a tumour-like increase at the site of formation, to a *plasmocytoma* (multiple myeloma, Kahler's disease, Figs. 151—154, 157 B—160), than to a flooding of the blood with these cells or *plasma cell leukaemia* (Fig. 150)

d) Disturbances in reproduction. Whereas rapid regeneration of the erythrocytes is almost invariably accompanied by an increase in the number of proerythroblasts, hyperplasia of the leucocytes can occur without an increased formation of blast cells, even in cases of leukaemia. The haemopoietic organs then exhibit marked proliferation of the intermediate stages—the promyelocytes and myelocytes—but there is no proliferation of the blast cells*. Thus in the eosinophilic leukaemia shown in Figs. 67 and 68, where 97 % of the cells in the bone marrow were eosinophils, the majority were promyelocytes and myelocytes and only very few were blast cells On the other hand, in the two cases of basophilic leukaemia shown (Figs. 45 A, 55 and 59), numerous blast cells were present, a picture similar to that often seen in neutrophilic leukaemias The behaviour of the different species of leucocytes with respect to regeneration is not uniform and varies from case to case.

In all cells which remain diploid during reproduction, the most minor visible disturbance of mitosis is that in which the nucleus divides but the cytoplasm does not, giving rise to polyploid cells, see pp 22 ff.

* Rohr K (1937) Knochenmarksmorphologie des menschlichen Sternalpunktates, *Klin Forth* 4, 498—564 (559)

A very rare phenomenon, seen in binuclear cells when the disturbance in proliferation is particularly profound, is dissociated karyokinesis, as shown in Fig. 32 C, where one nucleus is still in interphase (resting stage) while the other is already in prophase. Equally rare is dissociated destruction of the nuclei, as in Fig. 33 C, where one nucleus still has the vital structure and the other is undergoing premature destruction (necrobiosis).

The reappearance and proliferation of the megalocyte system in adults suffering from pernicious anaemia must be regarded as a special type of disturbance.

e) Disturbances in maturation and in the entrance of blood cells into the circulation. Owing to the close connection between the two processes involved, it is not always possible to separate these two disorders from one another.

During the course of various anaemias, the cytoplasm of the erythrocytes develops a basophilic stippling (Fig. 23) which is coarser than that sometimes observed in healthy individuals. In lead poisoning, stippling of the normocytes is very common. In anaemias, the erythrocytes may also exhibit basophilic loops and rings, the so-called Cabot's rings (Fig. 19 B).

Anomalies of the leucocytes are associated with peculiarities in maturation. In Alder's granulation anomaly, the neutrophils, eosinophils and basophils contain an unusually plentiful and coarse azurophilic granulation, as do also some of the monocytes and lymphocytes (Figs. 181 and 182). In Hegglin's polyphylic disturbance of maturation, strands of basophilic cytoplasm (Doehle's inclusion bodies) persist in the mature segmented leucocytes (Fig. 183) and the cytoplasm of the megakaryocytes gives rise only to abnormally large platelets (Fig. 184). In Pelger-Huet's anomaly, the nuclei of the blood cells are particulary dense, more so in the homozygotic manifestation (Figs. 77 A and 78 A) than in the heterozygotic manifestation (Figs. 77 B and 78 B). As a result, in homozygotic individuals the segmentation of the nucleus which normally occurs on maturation of the basophils, eosinophils and neutrophils is completely prevented, and in heterozygotic individuals only limited segmentation is possible. The contrary occurs in hereditary hypersegmentation of the neutrophil nuclei, the majority of the mature neutrophils containing four or five nuclear segments instead of the usual three (Fig. 80). In the hereditary increase in segmentation occurring in the eosinophil nuclei, most of the eosinophils have three nuclear segments instead of the usual two (Fig. 64 B).

Reactive and "primary" (leukaemic) changes in the leucocytes are due largely to disturbances in maturation and in the release of the blood cells into the circulation. The *toxic granulation* of the staff neutrophils and segmented neutrophils of the blood (Fig 76) is an azurophilic granulation which is very marked even in the early stages of development in the bone marrow (Fig 84) *Auer rods (Auer bodies)* are azurophilic crystals which may take the place of the azurophilic granules in the neutrophilic promyelocytes in certain leukaemias (Figs. 87 and 92). The persistence of *Doehle's inclusion bodies* is particularly liable to occur in acute infections (Fig. 75). *Vacuoles,* which are really gaps in the cytoplasm filled with chromophobic substances, are normally present only in plasma cells (Figs. 49 C, 144—146) but, under pathological conditions, they may occur in any species of blood cell (Figs. 85 A, 105, 106, 113—116 and 141). They should not be confused with similar "gaps" in the cytoplasm produced in bone marrow films by the presence of fat droplets (Figs 53, 54, 56 A, 56 C, 70 B, 72 A, 85 C, 86 A, etc) This fat, which is derived from ruptured stroma cells, is often taken up

by eosinophils (Fig. 65). *Abnormal indentation and segmentation of the nucleus*, occurring in young leucocytes which normally still have round nuclei, is particularly frequent in leukaemias. Such cells are usually known as "para" forms, e.g. "paramyeloblasts", but we prefer to use the term "atypical". Abnormalities of this type have been observed in neutrophils, eosinophils, basophils, monocytes, lymphocytes and plasma cells. Lymphocytes with deformed nuclei are known as *Rieder forms*

Cells in the juvenile stages of development, which do not normally appear in the blood, may enter the circulation in response to infections and poisoning as well as in leukaemic conditions, owing to the so-called "crowding-out" effect of the hyperplastic bone marrow. An increase in the number of staff neutrophils and juvenile neutrophils in the blood (Fig 75) is known as a *shift to the left* or *regenerative nuclear shift*. It should not be confused with the apparent shift to the left in the heterozygotic manifestation of Pelger's anomaly (Fig. 77B), which is without consequence. The release of immature eosinophils (Fig 64 D) and monocytes (Fig. 122 A) into the blood stream may also be the result of reactive processes, and even the normoblasts, down to the youngest known cell of the system, the pronormoblast, can appear in the circulation in such conditions (Fig. 22).

The opposite phenomenon, the so-called "shift to the right", a "primary" or reactive hypersegmentation of the nucleus, occurs less frequently. In pernicious anaemia, certain of the nuclei of the basophils, eosinophils, and neutrophils are highly segmented (Figs. 64 C and 81). Their precursors are "metamyelocytes" with particularly large, indented nuclei, so-called "giant metamyelocytes" (Figs 85 C and 86). The neutrophils may also very occasionally exhibit hypersegmentation of the nucleus, or a "shift to the right", as the result of infection (Fig. 82).

Under certain circumstances, blood cells which normally have round nuclei may develop nuclear segmentation or even hypersegmentation. This happens fairly often in the case of the erythroblasts in various anaemias (Figs. 23 C, 238 O and 238 P) but it may also occur in the lymphocytes and plasma cells (Figs. 148 C and 154). The normal segmentation of the nucleus which occurs in certain species of leucocytes, e.g. the neutrophils, is part of the process of maturation; as the cell grows older, the nucleus decreases in size and develops indentations, the denser, less easily absorbed portions gradually becoming almost completely separated. This segmentation begins shortly after the last mitosis when the cell is no longer able to proliferate. Within certain limits the number and appearance of the segments are typical for each species of segmented leucocyte. Under pathological conditions, segmentation of nuclei which otherwise remain round may occur, but the process is different from that just described. After the last mitosis, the chromosomes no longer reunite to form a round nucleus, but pass singly or in small groups into interphase ("resting phase"). The segments formed in this way are situated near the circumference of the cell and are connected by filaments. Such nuclei are referred to as "arrested mitoses". They may be recognized by the fact that they usually contain a large number of segments, frequently arranged in a rosette pattern (Fig. 154). As a rule, therefore, pathological segmentation of normally round nuclei is effected by quite a different process from that occurring in the nuclei of leucocytes which are normally segmented.

f) **Disturbances connected with the destruction of the blood cells.** The destruction of the blood corpuscles has so far been very little investigated, since, under normal conditions, it

takes place extremely rapidly and is not easy to observe. It terminates in the total liquefaction of the entire cell. Young cells in the process of destruction are found in leukaemias characterized by overproduction of immature cells (Figs. 131 B, 227 A, 228 G, 228 H, 229 B—229 D, 231 C). The occurrence of such disintegrating forms is a proof that these leukaemias are associated with pathological, very degenerate cells, which, despite their youth, are incapable of further maturation.

L. E. cells (Figs. 185, 186), found in patients suffering from acute disseminated lupus erythematosus, may be either neutrophils (Fig. 185) or monocytes (Figs. 186 D, 186 E) which have ingested the necrotic nuclei of neutrophils. The blood plasma of these patients contains a special factor which is able to induce the same behaviour in the blood cells of healthy individuals, even *in vitro*.

The *Howell-Jolly bodies* (Fig. 21 A) are nuclear remnants, for they give a positive Feulgen reaction which is specific for nuclear substance (Fig. 21 B). They are found in the erythrocytes in the blood in various anaemias and occur with particular frequency and regularity after splenectomy. Their formation cannot be regarded as physiological, since the nucleus of the erythroblasts, after becoming liquid at the still relatively large size of about 2—3 μ, is normally absorbed and broken down to Feulgen-negative substances without further reduction in size

Siderocytes (Grüneberg) (Figs. 19 D and 19 E) are erythrocytes containing granules which give a positive Prussian blue reaction for iron. They are probably senile cells and are only rarely seen, e. g. after splenectomy.

Heinz-Ehrlich bodies (Fig. 20) are break-down products which are formed in erythrocytes both *in vivo* and *in vitro* under the harmful influence of certain agents, particularly of antifebrin, aniline and the sulphonamides.

Blood parasites, see pp. 72—75 and Figs. 239—256.

6. CELLULAR ELEMENTS OCCURRING IN BONE MARROW FILMS BUT NOT BELONGING TO THE HAEMOPOIETIC SYSTEMS

Morphological haematology embraces both the true blood corpuscles and the cellular elements from which they are formed. The examination of the blood corpuscles is therefore not restricted to the blood but is also extended to their sites of formation, particularly the bone marrow obtained by puncture. Bone marrow films are prepared and examined in a similar manner to blood films, but they also contain cells which do not belong to the haemopoietic systems. Since it is of practical importance that these elements should be recognized, a short description of them is given here. The following types of cell may be encountered:

a) **Cells proper to the bones and bone marrow**

Basophils with insoluble granulation (tissue basophils), Figs 191 and 192.

Vascular cells (endothelial cells, muscle cells), Figs 193—196.

Stroma cells (fat cells, "reticulum cells"), Figs. 197—200 and 237.

Osteoblasts, Figs. 201 and 202.

Osteoclasts (polykaryocytes), Figs. 203—206.

In certain animals, the *basophils with insoluble granulation* appear in the circulation but, with the exception of a single case so far published*, they have not been found in the circulating blood in man. In the case described, the patient exhibited a benign but very marked increase in the number of tissue basophils, particularly in the skin. Apparently, they also passed into the blood stream, for the authors report that tissue basophils were found even in films prepared from blood obtained by venepuncture. They cannot, however, be classified as true blood cells in man. The formation of the tissue basophils takes place in the lymphatic tissue throughout the body. Not only do they originate in the same centres as the lymphocytes, monocytes and plasma cells but they are also similarly affected in various diseases, e.g. in panmyelopathy (Fig. 188).

The remaining cells listed above have no direct connection with the *formation of the blood* corpuscles, even in animals. — In the case of the *vascular cells*, differentiation between endothelial cells and muscle cells may be impossible, since usually only the naked nuclei remain. — The *stroma cells* are the only cells to which the term "reticulum cells" can really be applied, as they contain reticulin (Fig. 199) and are connected with one another by cytoplasmic processes to form a reticulum. However, they play no direct part in the formation of blood cells. They serve more as supporting tissue for the haemopoietic parenchyma, and as fat cells they store reserve fat and help to equalize the pressure in the medullary cavities of the bones. Cells of this type are widely distributed elsewhere in the organism where they also act as fat reservoirs and as supporting and interstitial tissue. *Osteoblasts* and *osteoclasts* are much more numerous in embryos, infants, children and young persons, that is, during the period of growth of the bones, than in adults. In osteoclast sarcomas the most bizarre cell formations may be seen (Figs. 207 and 208).

b) Cells foreign to the bones and bone marrow

Elements introduced as the result of pathological conditions: tumour cells, Figs. 209—220 and 232

Elements introduced during marrow puncture: cutaneous epithelial cells, epithelial cells of the sebaceous and sweat glands, hair follicles, hair fragments, basophils with insoluble granulation from the skin, vascular cells and stroma cells (fat cells) from the skin, Figs. 221—225.

Cells from other sources, particularly from saliva, Fig. 226

The recognition of tumour cells in the bone marrow is of great practical importance. They derive from tumours of the bones themselves or from the metastases of malignant growths. Elements introduced during marrow puncture have no special significance, but it is necessary to recognize them in order to avoid confusion with true bone marrow cells. This also applies to saliva cells, particularly to the nucleated, squamous epithelium, containing bacteria from the buccal cavity, (Fig. 226) which may be expelled on to the preparation with droplets of saliva during speaking.

* Hissard, R., Moncourier, L. and Jacquet, J. (1950): "Une nouvelle affection hématodermique, la mastocytose", *C. R. Soc. Biol.* 231, 153
— — — (1950): "A propos d'un cas de mastocytose pure", *C. R. Soc. Biol.*, 231, 1178

Table 3

FORMATION OF DIPLOID AND POLYPLOID CELLS

Mitoses, endomitoses, giant chromosomes

Regular forms*

A B C D

diploid · diploid · tetraploid · polyploid · diploid · octoploid · etc.

In order to simplify the diagram, only 8 chromosomes have been shown instead of the 48 found in man The normal diploid nuclei of the interphases (resting nuclei) are depicted with 2 nucleoli.

Column A: Formation of diploid cells.

Columns B and C· Formation of polyploid mononuclear and polynuclear cells, accompanied by division of the chromosomes

Column D. Formation of polyploid mononuclear cells with giant nucleoli; doubling of the chromosomes without division.

1—9 Interphases (resting stages)

1 Diploid cells.
2 Tetraploid cell with two diploid nuclei (Figs 33, 39 A, 69 B, 69 C, 102, 103, 105 A, 126, 127 A, 138 A, 156 A, 157 A, 158 A above and 162 D)
3 Tetraploid cell with one tetraploid nucleus and normal nucleoli (Figs 127 B, 127 C, 158 A below and 162 E)
4 Tetraploid cell with one tetraploid nucleus and giant nucleolus
5 Octoploid cell with 4 diploid nuclei (Figs 159 left and 163 F)
6 Octoploid cell with 2 diploid nuclei and one tetraploid nucleus (Figs 105 B and 159 right)
7 Octoploid cell with 2 tetraploid nuclei.
8 Octoploid cell with one octoploid nucleus and normal nucleoli (Fig 163 G)
9 Octoploid cell with one octoploid nucleus and giant nucleolus (Figs 35 D and 36 E)

*a—h Mitoses and endomitoses in anaphase***.

a Normal, bipolar anaphases of diploid cells (Figs. 32 B, 38 C, 98 C, 100 C, 136 G and 155 B)
b Endomitosis of a diploid cell with divided chromosomes
c [illegible]
d [illegible]
e [illegible] chromosomes
f Bipolar endomitosis of a tetraploid cell with divided chromosomes
g Monopolar endomitosis of a tetraploid cell with divided chromosomes (Fig 164 H)
h Monopolar endomitosis of a tetraploid, mononuclear cell with giant chromosomes division of the chromosomes having failed to take place (Fig 35 C)

* The commonly occurring irregular forms (p. 24) are not shown here, since they may be easily deduced from the regular forms

** The degree of polyploidy and phase of mitosis exhibited by the examples given in brackets do not always correspond with those shown in the diagram above, and in some cases irregularities are also present Nevertheless, the examples illustrate the characteristic features



7. POLYPLOIDY OF THE BLOOD CELLS

In haematology a knowledge of the polyploid elements is of particular importance since some of the blood cells undergo polyploid development even under normal conditions*, while under pathological conditions all the remaining species may exhibit polyploidy**.

a) Terminology

Haploid ([illegible] = single) is used to describe cells containing only a *single* set of chromosomes (in man 24 chromosomes). The male and female germ cells are haploid following meiosis.

Diploid ([illegible] = double) describes cell nuclei or cells possessing a *double* set of chromosomes (in man 48 chromosomes). The fertilized germ cell is diploid since it arises from the fusion of a haploid male gamete with a haploid female gamete. All the somatic cells derived from it are likewise diploid as long as karyokinesis is accompanied by division of the nucleus and cytoplasm.

Heteroploid ([illegible] = other) is a term introduced by Winkler in 1916 to describe cell nuclei or cells, the chromosome number of which is other than diploid. The chromosome number may be increased or diminished.

Polyploid ([illegible] = many) is Winkler's term for cell nuclei or cells containing more than two sets of chromosomes. Such cells may be *tetraploid* (four sets of chromosomes), *hexaploid* (6 sets), *octoploid* (8 sets) *16 ploid* (16 sets), etc. Although according to the classical conception the term "polyploidy" refers only to nuclei and cells with chromosome numbers which are integral multiples of the haploid number, it has subsequently been extended to include cells containing more than two sets of chromosomes, even when some of the sets are incomplete. Owing to the impossibility of determining the exact number of chromosomes in the blood cells in man, the term "polyploid" must also be used in the wider sense in haematology.

Adopting the nomenclature proposed by Täckholm in 1922, polyploid elements may be subdivided into "euploid" and "aneuploid" cells. They are *euploid* ([illegible] = well) when the chromosome number is an integral multiple of the haploid number. If the complement of chromosomes includes an incomplete set in addition to one or more complete sets, the cell is termed *aneuploid* ([illegible] = un[illegible]). Aneuploid elements may be hyperploid or hypoploid (Winkler, 1916). They are *hyperploid* if the chromosome number is slightly increased, e.g., hypertetraploid, hyperhexaploid. If the number of chromosomes is slightly decreased, the cells are termed *hypoploid*, e.g., hypotetraploid, hypohexaploid. The latter phenomenon may be exhibited by diploid as well as by polyploid nuclei, the cells being then described as *hypodiploid*.

Polyploid cells are formed when the cytoplasm of the cell remains undivided during karyokinesis. The nucleus may also remain undivided and simply double in size (endomitosis), or mitotic division of the nucleus may occur resulting in the formation of a polyploid cell with two nuclei. After further karyokineses this binuclear cell gives rise to cells containing three, four or more nuclei (e.g. osteoclasts). *Polyploid nuclei* are produced when the nucleus as well as the cytoplasm remains undivided and simply doubles in size (e.g. megakaryocytes). This may occur either as a result of fusion of two or more poles during karyokinesis or when karyokinesis takes place without spindle formation. For the latter so-called "internal mitosis" Geitler (1938) introduced the term "endomitosis". During endomitosis the nuclear membrane remains intact whereas this is not the case when true mitosis takes place. Since the nuclear membrane of the blood cells is not visible, it is impossible in haematological work to decide by which mechanism the polyploid nucleus has been produced. A polyploid cell may contain a single large polyploid nucleus, several diploid or polyploid nuclei, or both diploid and polyploid nuclei, the total number of chromosome sets corresponding to the degree of polyploidy of the cell.

* Rothlin, E. and Undritz, E. (1946) "Zur Megakaryozytenbildung durch Polyploidie". *Arch. Klaus-Stift. Vererb. Forsch. 21*, 283.

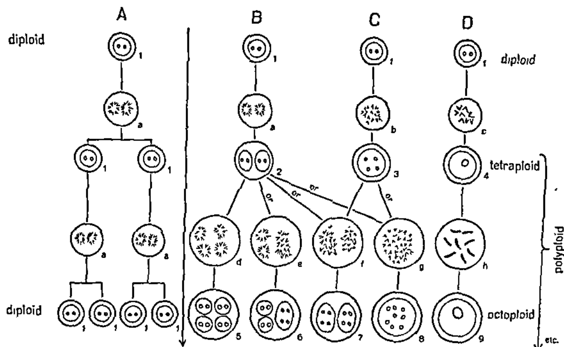
** Undritz, E. (1951) "Pathological Polyploidy (Heteroploidy) of the Blood Cells and its Significance in Leukaemia". *Proc. Int. Soc. Hematol.*, 3, 184.

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Column D. Formation of polyploid mononuclear cells with giant nucleoli; *doubling of the chromosomes without division*

1—9· Interphases (resting stages)

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4. *Tetraploid* cell with one tetraploid nucleus and giant nucleolus
5. Octoploid cell with 4 diploid nuclei (Figs. 159 left and 163 F).
6. *Octoploid cell with 2 diploid* nuclei and one tetraploid nucleus (Figs 105 B and 159 right)
7. Octoploid cell with 2 tetraploid nuclei.
8. Octoploid cell with one octoploid nucleus and normal *nucleoli (Fig 163 G)*
9. Octoploid cell with one octoploid nucleus and giant nucleolus (Figs 35 D and 36 E).

*a—h Mitoses and endomitoses in anaphase**.*

- a Normal, bipolar anaphases of diploid cells (Figs. 32 B, 38 C, 98 C, 100 C, 136 G and 155 B)
- b Endomitosis of a diploid cell with divided chromosomes
- c [illegible]
- d [illegible]
- e Tripolar anaphase, combined mitosis and endomitosis of a tetraploid, binuclear cell with divided chromosomes
- f Bipolar endomitosis of a tetraploid cell with divided chromosomes
- g Monopolar endomitosis of a tetraploid cell with divided chromosomes *(Fig 164 H)*
- h *Monopolar endomitosis of a tetraploid, mono*nuclear cell with giant chromosomes, division of the chromosomes having failed to take place (Fig 35 C)

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** The degree of polyploidy and phase of *mitosis exhibited* by the examples given in brackets do not always correspond with those shown in the diagram above, and in some cases irregularities are also present Nevertheless, the examples illustrate the characteristic features

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6 [illegible] tetra- [illegible]
7 [illegible]
8 [illegible] is and [illegible]
9 Octoploid cell with one octoploid nucleus and giant nucleolus (Figs 35 D and 36 E)

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Adopting the nomenclature proposed by Täckholm in 1922, polyploid elements may be subdivided into "euploid" and "aneuploid" cells They are *euploid* (εὖ = well) when the chromosome number is an integral multiple of the haploid number. If the complement of chromosomes includes an incomplete set in addition to one or more complete sets, the cell is termed *aneuploid* (ἄν = un) Aneuploid elements may be hyperploid or hypo-

the cells being then described as *hypodiploid*

Polyploid cells are formed when the cytoplasm of the cell remains undivided during karyokinesis The nucleus may also remain undivided and simply double in size (endomitosis), or mitotic division of the nucleus may occur resulting in the formation of a polyploid cell with two nuclei After further karyokineses this binuclear cell gives rise to cells containing three, four or more nuclei (e g osteoclasts) *Polyploid nuclei* are produced when the nucleus as well as the cytoplasm remains undivided and simply doubles in size (e g megakaryocytes) This may occur either as a result of fusion of two or more poles during karyokinesis or when karyokinesis takes place without spindle formation For the latter so-called "internal mitosis" Geitler (1938) introduced the term "endomitosis" During endomitosis the nuclear membrane remains intact whereas this is not the case when true mitosis takes place Since the nuclear membrane of the blood cells is not visible, it is impossible in haematological work to decide by which mechanism the polyploid nucleus has been produced A polyploid cell may contain a single large polyploid nucleus, several diploid or polyploid nuclei, or both diploid and polyploid nuclei, the total number of chromosome sets corresponding to the degree of polyploidy of the cell

* Rothlin, E. and Undritz, E. (1946) "Zur Megakaryozytenbildung durch Polyploidie". *Arch Klaus-Stift Vererb.-Forsch* 21, 283

** Undritz, E (1951). "Pathological Polyploidy (Heteroploidy) of the Blood Cells and its Significance in Leukaemia" *Proc Int Soc Hematol*, 3, 284

Table 3

FORMATION OF DIPLOID AND POLYPLOID CELLS

Mitoses, endomitoses, giant chromosomes

Regular forms*

In order to simplify the diagram, only 8 chromosomes have been shown instead of the 48 found in man. The normal diploid nuclei of the interphases (resting nuclei) are *depicted with 2 nucleoli.*

Column A: Formation of diploid cells.

Columns B and C Formation of polyploid mononuclear and polynuclear cells, accompanied by division of the chromosomes

Column D: Formation of polyploid mononuclear cells with giant nucleoli; doubling of the chromosomes without division.

1—9· Interphases (resting stages)

1 Diploid cells.
2 Tetraploid cell with two diploid nuclei (Figs. 33, 39 A, 69 B, 69 C, 102, 103, 105 A, 126, 127 A, 138 A, 156 A, 157 A, 158 A above and 162 D)
3 Tetraploid cell with one tetraploid nucleus and normal nucleoli (Figs. 127 B, 127 C, 158 A below and 162 E).
4 Tetraploid cell with one tetraploid nucleus and giant nucleolus
5 *Octoploid cell with 4 diploid nuclei* (Figs 159 left and *163 F)*
6 Octoploid cell with 2 diploid nuclei and one tetraploid nucleus (Figs 105 B and 159 right)
7 Octoploid cell with 2 *tetraploid nuclei*
8 Octoploid cell with one octoploid nucleus and normal nucleoli (*Fig 163 G)*
9 Octoploid cell with one octoploid nucleus and giant nucleolus (Figs 35 *D* and *36 E)*

*a—h. Mitoses and endomitoses in anaphase***.*

a Normal, bipolar anaphases of diploid cells (Figs. 32 B, 38 C, 98 C, 100 C, 136 G and 155 B).
b Endomitosis of a diploid cell with divided chromosomes.
c [illegible]
d [illegible]
e [illegible] chromosomes
f Bipolar endomitosis of a tetraploid cell with divided chromosomes
g Monopolar endomitosis of a tetraploid cell with divided chromosomes (Fig 164 H)
h Monopolar endomitosis of a tetraploid, mononuclear cell with giant chromosomes division of the chromosomes having failed to take place (Fig 35 C)

* The commonly occurring irregular forms (p. 24) are not shown here, since they may be easily deduced from the regular forms

** The degree of polyploidy and phase of mitosis exhibited by the examples given in brackets do not always *correspond* with those shown in the diagram above, and in some cases irregularities are also present. Nevertheless, the examples illustrate the characteristic features

7. POLYPLOIDY OF THE BLOOD CELLS

In haematology a knowledge of the polyploid elements is of particular importance since some of the blood cells undergo polyploid development even under normal conditions*, while under pathological conditions all the remaining species may exhibit polyploidy**.

a) Terminology

Haploid (ἁπλοῦς = single) is used to describe cells containing only a *single* set of chromosomes (in man 24 chromosomes) The male and female germ cells are haploid following *meiosis*

Diploid (διπλοῦς = double) describes cell nuclei or cells possessing a *double* set of chromosomes (in man 48 chromosomes) The fertilized germ cell is diploid since it arises from the fusion of a haploid male gamete with a haploid female gamete. All the somatic cells derived from it are likewise diploid as long as karyokinesis is accompanied by division of the nucleus and cytoplasm

Heteroploid (ἕτερος = other) is a term introduced by Winkler in 1916 to describe cell nuclei or cells, the chromosome number of which is other than diploid. The chromosome number may be increased or diminished

Polyploid (πολύς = many) is Winkler's term for cell nuclei or cells containing more than two sets of chromosomes Such cells may be *tetraploid* (four sets of chromosomes), *hexaploid* (6 sets), *octoploid* (8 sets) *16-ploid* (16 sets), etc. Although according to the classical conception the term "polyploidy" refers only to nuclei and cells with chromosome numbers which are integral multiples of the haploid number, it has subsequently been extended to include cells containing more than two sets of chromosomes, even when some of the sets are incomplete. Owing to the impossibility of determining the exact number of chromosomes in the blood cells in man, the term "polyploid" must also be used in the wider sense in haematology.

Adopting the nomenclature proposed by Täckholm in 1922, polyploid elements may be subdivided into "euploid" and "aneuploid" cells They are *euploid* (εὖ = well) when the chromosome number is an integral multiple of the haploid number If the complement of chromosomes includes an incomplete set in addition to one or more complete sets, the cell is termed *aneuploid* (ἄν = un) Aneuploid elements may be hyperploid or hypoploid (Winkler, 1916). They are *hyperploid* if the chromosome number is slightly increased, e g., hypertetraploid, hyperhexaploid If the number of chromosomes is slightly decreased, the cells are termed *hypoploid*, e g., hypotetraploid, hypohexaploid. The latter phenomenon may be exhibited by diploid as well as by polyploid nuclei, the cells being then described as *hypodiploid*

Polyploid cells are formed when the cytoplasm of the cell remains undivided during karyokinesis. The nucleus may also remain undivided and simply double in size (endomitosis), or mitotic division of the nucleus may occur resulting in the formation of a polyploid cell with two nuclei. After further karyokineses this binuclear cell gives rise to cells containing three, four or more nuclei (e g osteoclasts). *Polyploid nuclei* are produced when the nucleus as well as the cytoplasm remains undivided and simply doubles in size (e g megakaryocytes) This may occur either as a result of fusion of two or more poles during karyokinesis or when karyokinesis takes place without spindle formation. For the latter so-called "internal mitosis" Geitler (1938) introduced the term "endomitosis" During endomitosis the nuclear membrane remains intact whereas this is not the case when true mitosis takes place Since the nuclear membrane of the blood cells is not visible, it is impossible in haematological work to decide by which mechanism the polyploid nucleus has been produced A polyploid cell may contain a single large polyploid nucleus, several diploid or polyploid nuclei, or both diploid and polyploid nuclei, the total number of chromosome sets corresponding to the degree of polyploidy of the cell

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b) Polyploidy under normal conditions

The following blood cells are normally diploid: normoblasts, megaloblasts, basophils, eosinophils, neutrophils, monocytes, lymphocytes and plasma cells. The megakaryocytes are normally polyploid. Among the non-haemopoietic elements of the bone marrow encountered during haematological examinations, the only cells normally polyploid are the osteoclasts.

Since in man it is not possible to determine with certainty the number of chromosomes in the blood cells, *it is usually considered sufficient to distinguish only between diploid and polyploid elements.* If the cells are not too highly polyploid, it may also be possible to determine approximately the degree of polyploidy (tetraploid, or octoploid).

In deciding whether a blood cell is polyploid or not, the principal criterion is the appearance of the nucleus, the size of the cell being only of secondary importance During mitosis any appreciable excess of chromosomes becomes readily apparent and enables the cell to be recognized as "polyploid", e g., compare Fig 128 B with Fig. 125 B. For nuclei in interphase ("resting stage") the *number* and *size of the nuclei* are the deciding factors, but it must be remembered that the size of the nuclei depends to a great extent upon the degree of development of the cell. As far as the size of the cell as a whole is concerned, polyploid cells are larger than normal diploid cells, except when the latter have been engaged in phagocytosis or storage. They may then attain the size of polyploid cells, as happens when the vacuoles of the stroma or fat cells of the bone marrow contain much fat (Figs. 197—200) or when monocytes have consumed large amounts of foreign matter (Figs. 116, 118 B and 119—121).

In botany and in zoology *it is possible to distinguish not only polyploid nuclei and cells, but also polyploid* tissues, organs and individuals. The haematologist is concerned only with polyploid nuclei, cells and cell species (megakaryocytes and osteoclasts). The cells are designated according to the total number of chromosome sets which they contain, irrespective of whether the chromosomes are present in a single nucleus or distributed among several nuclei If a cell is polyploid and mononuclear, the degree of polyploidy of the nucleus is the same as that of the cell, e g., a tetraploid megakaryoblast with a single tetraploid nucleus (Fig. 162 E) or an octoploid megakaryoblast with a single octoploid nucleus (Fig. 163 G). If the cell is polyploid and polynuclear, the degree of polyploidy of the cell as a whole is given first, followed by the number and degree of polyploidy of the individual nuclei, e g., tetraploid megakaryoblast with two diploid nuclei (Fig 162 D), octoploid megakaryoblast with four diploid nuclei (Fig. 163 F), or with two diploid nuclei and one tetraploid nucleus, or with two tetraploid nuclei. These various possibilities are shown *schematically* in Table 3, columns A, B and C (*page 10*)

Although the characterization of polyploid cells meets with many difficulties and the finer differentiation of the sub-groups is seldom possible in the case of blood cells, these shortcomings do not detract in any way from the fundamental value of the classification It is important to know that under normal conditions one species of blood corpuscle, the megakaryocyte, and one species of non-haemopoietic cell of the bone marrow, the osteoclast, are polyploid elements.

c) Polyploidy under pathological conditions; atypical mitoses and nuclear anomalies

In pathological processes, abnormalities in the division of the blood corpuscles frequently occur, even when the *disease does not affect* the blood cells directly. Thus, in all species of blood cells which normally remain diploid, a certain number of polyploid elements may be found under pathological conditions. It is frequently *possible* to observe polyploid normocytes (Figs 22, 32 C, 33—36, 238 O and 238 P), megalocytes (Figs 39—41), basophils (Figs. 59 bottom left and 60 D), eosinophils (Figs 69 B, 69 C and 70), neutrophils (Figs 102—106), monocytes (Figs 117 B, 126—130 and 255), lymphocytes (Figs 139—142), and plasma cells (Figs 156—160) In tumour metastases or in tumours of the haemopoietic organs, polyploid cells are very common (Figs 211, 215 and 216—220) Pathological conditions may also cause changes in the type of polyploidy in those species of cells of the blood and haemopoietic organs which *are normally polyploid* For example, the megakaryocytes which normally have a single polyploid giant nucleus may become polynuclear (Figs 171 and 172) In the osteoclasts, which normally have many diploid nuclei, some polyploid nuclei may also appear (Figs 206—208) Occasionally,

unusually large binuclear megakaryocytes ("twinning deformities") may be formed, containing two highly polyploid nuclei, each of normal size, and sometimes arranged as mirror images of one another (Fig 178 A)

In principle, the formation of pathological polyploid elements is similar to that of normally polyploid cells, as can be seen from the schematic presentation in Table 3, columns A, B and C (p 20) In column D, on the other hand, is shown the development of polyploidy by a process so far observed only under pathological conditions In this case, not only the cytoplasm and the nuclei remain undivided during karyokinesis, but also the chromosomes With every karyokinesis, each of the chromosomes doubles in size but the number of chromosomes remains unchanged. Apparently connected with this phenomenon is the formation of enormously large, usually solitary nucleoli, which may reach the size of diploid blood cells (Figs. 35 D and 36 E) Under normal conditions, the degree of polyploidy can be determined approximately by means of the chromosome number, but even this criterion fails in the case of cells with giant chromosomes The degree of polyploidy can then be estimated only on the basis of the quantity of chromatin and the size of the cell

The most easily recognized abnormality of cell division is the failure of the cytoplasm to separate, leading to the formation of a tetraploid cell with two diploid nuclei—a twinning deformity. Elements of this type may occur even in healthy individuals and under conditions of normal haematopoiesis Especially likely to be found are tetraploid binuclear neutrophils (Fig 103) and lymphocytes (Fig 138) in the blood, and tetraploid binuclear plasma cells (Figs 156 A and 157 A) and normoblasts in the bone marrow. If haematopoiesis is stimulated by pathological processes, polyploid elements appear in larger numbers The degree of polyploidy may attain high values. Pathological polyploidy can be caused both by benign and by malignant diseases, and may be due either

are also polyploid elements

Pathological polyploid cells containing single giant nuclei are also known as "monsters" (Figs 35 D, 36 E, 127 B, 127 C, 128 C, 142 C, 142 D and 158 A).

Under pathological conditions it is not usual to find only a single type of polyploidy, but rather the simultaneous occurrence of various types (polynuclear cells with diploid nuclei, polyploid mononuclear cells and mixed forms) as in the case depicted in Figs 34—36

Large polyploid cells are not found in blood films since they are unable to reach the peripheral circulation. They may be observed, however, in preparations prepared by puncture of the haemopoietic organs, and can be detected most readily using low power magnification This needs a little practice, but the effort is worth while

Hypodiploid cells have not, so far, been detected with certainty in blood preparations The so-called micromyeloblasts have diploid nuclei and appear small mainly because they contain little cytoplasm Mature "microneutrophils", "microlymphocytes", etc, which are occasionally described are fragments of whole cells

They may result either from the formation of supernumerary poles or from the separation of small or large groups of chromosomes during mitosis, the number of nuclei formed being greater than that expected from the chromosome number (Figs 40 E, 138 B, 138 D, 160 B and 207) Thus, the complement of chromosomes in the binuclear lymphocyte in Fig 138 B is apparently diploid, and the same applies to the chromosome number of the

two small upper nuclei of the pentanuclear cell in Fig 40 E, which is therefore probably not more than octoploid The tetranuclear lymphocyte in Fig 138 D is probably only tetraploid, although it possesses four hypodiploid nuclei.

Uneven distribution of the chromosomes leads to the production of nuclei of unequal size, one nucleus being hyperdiploid, the other hypodiploid An example of this is to be seen in the lymphocyte in Fig 138 C, although the difference in size here is not very marked. In Fig 160 B each of the three pairs of nuclei apparently consists of a hyperdiploid and a hypodiploid nucleus joined together. Here the difference in size is more pronounced.

The term *heteroploid cells* can be used when hypodiploid, diploid and polyploid nuclei are lying side by side, the nature of the individual nuclei being practically impossible to determine, as in Figs *139, 140 and 207*

Chromosome bridges between the poles of cells in mitosis (Fig 40 D) lead to the formation of *nuclear bridges* of varying widths (Figs 40 E, 138 C, 138 D, 139, 140 and 238 O).

Isolated detached chromosomes (Fig 41 A) are not viable and form small, structureless, necrobiotic spheres, which may be considered as premature *Howell-Jolly bodies.* These are seen lying alongside the living nucleus, which is composed of the numerous remaining chromosomes and exhibits well-defined structure (Figs. 4 left and 41 B) The method of formation of these Howell-Jolly bodies, produced direct from isolated chromosomes, is different from that of the well-known Howell-Jolly bodies of the denucleated erythrocytes (Figs. 4 right and 21) The latter, which are seen, for example, following splenectomy, are apparently the end products resulting from reduction in size of the entire nucleus

The atypical mitoses and nuclear anomalies mentioned above are often combined with pathological polyploidy giving rise to *irregular polyploid forms* (in the diagram on p 20 only the regular forms are shown)

Bridge formation between nuclei and even polyploid binuclear and polynuclear cells were previously held to be evidence of incomplete *amitoses*. The true interpretation of these atypical cells became possible only when the technique of puncture was applied to the haemopoietic organs. This led to the discovery of the corresponding chromosome bridges and other abnormalities of karyokinesis, from which it became apparent that such deformities are invariably of mitotic origin As the duration of the mitotic stage is considerably shorter than that of the interphase, atypical nuclei are encountered much more frequently during the interphase ("resting nuclei") than during mitosis, when they are easily overlooked unless carefully searched for No convincing evidence exists that amitoses do occur in the case of the blood corpuscles (Undritz, 1944).

From the foregoing it is evident that even the most severe disturbances in karyokinesis may occur in benign diseases as reactive manifestations just as readily as in primary malignant diseases, such as leukaemias and tumours. Thus the highly polyploid, mononuclear normoblasts with giant chromosomes and giant nucleoli in Figs 35 C, 35 D and 36 E—cells with the most severe disturbances of karyokinesis imaginable—are only products of a benign disease. On the other hand, the Sternberg giant cells in Figs 217, 218 B and 220, which exhibit equally severe disturbances, are products of a malignant disease *Neither the occurrence of polyploid elements nor the severity of the disturbance in karyokinesis is in itself evidence of malignancy* The constant occurrence of highly polyploid elements is very suspicious, but its significance can only be properly assessed when taken in conjunction with the entire clinical picture It must also be remembered that malignant proliferative diseases may occur in the absence of pathological polyploidy.

As in the case of normal polyploidy, the difficulties encountered in making a precise classification of polyploidy under pathological conditions in no way detract from its significance. It is important to know that every species of cell which normally undergoes diploid development can produce polyploid cells under pathological conditions, and that even the most severe degrees of polyploid deformation may occur in benign affections as well as in [illegible] *made, therefore, merely from* [illegible] *opment.*

II. The Technique of Blood and Bone Marrow Examination

In the majority of cases in which a blood examination is necessary, sufficient information can be obtained by determining the sedimentation rate, the haemoglobin content, the absolute erythrocyte and leucocyte counts and the relative blood picture. Other blood determinations and bone marrow puncture may be carried out in special cases where additional information is required. Since this atlas is concerned principally with the morphological characteristics of the blood, only the technique of making blood films and the methods of staining blood and bone marrow films will be considered. For descriptions of other than morphological blood examinations and the technique of marrow puncture, as well as for the rarely required techniques of lymph gland and splenic puncture, the reader is referred to the relevant manuals on these subjects.

After making a bone marrow puncture, about 10 films should be prepared and several of these reserved for any special staining which may later be found necessary. Before giving the local anaesthetic, it is advisable to make a blood examination, or at least to prepare a few blood films, for comparison with the bone marrow findings.

Of the large number of methods available for the examination of the blood corpuscles, only those will be mentioned here which we have found particularly satisfactory and which enable all the known cells to be differentiated and identified. For the preparation of blood films, the use of microscope slides is preferred (*Schweiz. med. Wschr. 1944, 74, 995*). The instructions for staining are therefore intended for slide preparations; if coverslip preparations are used, the staining technique must be modified accordingly.

It is recommended that the following general directions be adhered to.

Whenever possible, the *removal of the blood sample* should be carried out while the patient is still in bed and before food has been taken (preferably before the usual breakfast time). The ingestion of food, or fasting beyond the usual breakfast time, may cause a leucocytosis. In urgent cases, blood examinations may have to be carried out at any time of the day, but variations in the counts must then be expected.

The freshly prepared films should be air-dried at room temperature and all forms of warming, even exposure to the sun, should be avoided. If the air is damp, drying can be accelerated by waving the slides. Until fixation, they should be preserved with the preparation side upwards, preferably in a drawer, protected from all possible injury. It takes several hours before drying is complete and panoptic staining should therefore be postponed for at least 4—5 hours, and preferably for 24 hours. In urgent cases, one film may be stained immediately, however, and a second one set aside for staining later. Before and after the individual staining procedures, the slides can be stood on a sheet of blotting paper resting against the edge of a soup plate or other suitable object, and left to dry with the preparation side facing outwards. Direct drying with blotting paper seriously damages the preparation. Even after staining, the preparation should not be warmed.

With the exception of the stain (l), the preparations should *not be covered* with Canada balsam and cover slips, as Canada balsam rapidly bleaches the dyes. After examination, the cedar oil is removed from the slide with a wad of cotton wool soaked in xylene, fresh cotton wool being used each time. Cloths or tissue paper, as well as wads of cotton wool which have already been used once, are liable to scratch the preparations. For the preservation of preparations stained with Sudan III, see under Staining Method (n).

For marking individual groups of cells, a simple mounted needle may be used if no special marking apparatus is available. Using low-power magnification, a ring is drawn round the cells in question by scratching the surface of the film with the needle. With the aid of a reading glass, a somewhat larger circle is then drawn in Indian ink at the corresponding place on the back of the preparation.

For storage purposes, the preparations which have been examined are freed from cedar oil, stacked one above the other and wrapped in a strip of smooth writing paper on which can be noted any relevant information. Slides protected in this way may be simply stored in a cardboard box. In comparison with the use of special storage cabinets, this procedure has the advantages that it saves space and that the preparations are better preserved as they are kept more air-tight. Blood preparations should not be destroyed as they form valuable objective documents.

1. Technique of making blood films on microscope slides.

Necessary equipment: microscope slide, spreader slide.

Preparation of slides: The microscope slides must be scrupulously clean. If needed at short notice, it is sufficient to rub the dirty slide on both sides with the edge of a piece of soap and then to wash it thoroughly under running water with freshly washed hands, afterwards drying with a clean towel. It is preferable, however, to keep a stock of clean slides. For this purpose, the slides are placed for 24 hours in 10 % caustic potash or in a mixture of chromic acid and sulphuric acid, rinsed for several hours in running water and then dipped first in 95 % alcohol and afterwards in ether. After drying with a clean linen cloth, the slides are polished with a clean chamois leather, wrapped in groups of 5 or 10 in thin, fibre-free writing paper, and stored in a box protected from dust. *Clean slides must only be held by their edges and the fingers must never touch the sur-*

faces. As a spreader, a clean cover glass from a counting chamber or another slide may be used. The edges must be ground smooth, however, and a corner should be broken off at each end to ensure that the blood film remains "margin-free", i e., does not reach to the edges of the slide.

Procedure. The finger tip or lobe of the ear is cleaned with ether and punctured with a Francke's needle or, should one of these not be available, with a syringe needle or a lancet. The first drop of blood is discarded The top of a succeeding drop, not too large in size, is quickly transferred to a slide, near to one end, taking care that the glass does not touch the finger and thus smear the drop The slide is then held with the drop on the right, placed on a solid surface (table) and the left hand end grasped firmly between the index finger and the thumb of the left hand The spreader glass is taken in the right hand, held at an angle of 45° and stroked across the slide from left to right until in contact with the drop of blood The blood spreads along the edge of the spreader which is then slid evenly, neither too slowly nor too rapidly, from right to left so that the blood is drawn after it and spread out to form a thin film This technique is preferable to that of pushing the drop forwards, which results in many cells being damaged The drop is of the correct size if the film does not reach the left hand end of the slide Short, thin films are more useful than long, thick ones, and they should be "margin-free". For each examination, at least three good films must be prepared. After drying, the name of the patient and the date are written in pencil on the thickest part of the preparation

The most suitable part of the film for microscopic examination is that where the erythrocytes lie close to one another but not one above the other This is usually near the end of the film. The extreme ends and the edges are unsuitable for purposes of differentiation as they contain densely packed large cells which are often damaged. Nevertheless, it is advisable to inspect these areas under the low power and to subject any suspicious elements to closer examination under oil immersion

2. Staining of blood and bone marrow films.

If the diagnosis is unknown, at first only one blood or bone marrow film is stained, using either Giemsa's, Pappenheim's or Wright's stain, all of which are suitable for general differentiation The relative merits of these three panoptic methods of staining will be discussed later; in principle, one should suffice to establish the diagnosis If the patient is anaemic, a second blood preparation is stained with Hirschfeld's stain for determination of the proerythrocytes (reticulocytes) If differentiation of the blood picture is difficult, additional information may be obtained from the Graham-Knoll peroxidase reaction, which is particularly advisable in all cases of leukaemia The remaining blood preparations can be used for any special staining which is found to be necessary.

Table 4

SUMMARY OF THE STAINING METHODS DESCRIBED AND THEIR APPLICATIONS

Staining method	Application
a) Giemsa; b) Pappenheim; c) Wright } panoptic staining	General differentiation
d) Brilliant cresyl blue (Wolfer)	*Proerythrocytes (reticulocytes)*
e) Hirschfeld	" "
f) *Peroxidase reaction* (Graham-Knoll)	General peroxidase differentiation
g) Peroxidase reaction (Lepehne)	Erythroblasts
h) Modified peroxidase reaction I (Undritz)	Monocytes
i) Modified peroxidase reaction II (Undritz)	Eosinophils
k) Toluidine blue (Undritz)	Basophils
l) Silver impregnation (Gömöri)	Stroma cells of the bone marrow
m) Nile blue sulphate	Heinz-Ehrlich bodies
n) Sudan III (Romeis)	Fat
o) Sudan black B (Lison)	As for Graham-Knoll peroxidase reaction
p) Feulgen	Nuclear substance
q) Prussian blue reaction (Grüneberg)	Siderocytes and sideromonocytes
r) Thick drop (Schilling)	Blood parasites

a) Giemsa's stain.

Necessary reagents

i) *Methyl alcohol,*

ii) *Giemsa's stock solution*

1 Fixation of the air-dried preparation for 15 minutes in methyl alcohol, preferably by dipping into a staining jar filled with methanol rather than pouring the alcohol over the preparation If contamination with water is avoided, the methyl alcohol may be used several times The containers should, therefore, be kept covered Dry the preparation without rinsing

2 Staining.

a) For 1—2 preparations: 0 3 ml (10 drops) Giemsa solution in 10 ml distilled water Lay the slides, film side downwards, on match sticks or glass rods in a Petri dish, and introduce the staining solution underneath

b) For large numbers of slides 3—4 ml Giemsa solution in 100 ml distilled water. Hellendahl's dish is suitable for up to 23 preparations Two slides are placed back to back in each of the eight compartments and a further slide is stood obliquely in each of the seven intervening spaces The staining solution is then poured in

The distilled water must be boiled, cooled, and kept in a closed vessel (free from CO_2) The Giemsa solution is measured into a measuring cylinder, the water added rapidly and the two mixed by tilting two or three times It is advisable to pour the mixture into the Petri dish or Hellendahl's dish through a funnel containing a little cotton wool to remove the iridescent skin Leave for 15—30 minutes, depending upon the intensity of the staining solution. The latter is then washed away with tap water. If the staining is too weak, the operation must be repeated Dry as described on p 25

Result In the leucocytes, the cells with basophilic (blue) cytoplasm can be readily distinguished from those with acidophilic (pink) cytoplasm The cytoplasm stains *blue* in mature *lymphocytes, plasma cells* and *monocytes* (Fig 44) and pink in mature *basophils, eosinophils* and *neutrophils* (Fig 43) *Monocytes* with indented nuclei are therefore easily distinguished from *juvenile* [illegible]

[illegible]

++ or +++ The granulation of the *basophils* usually washes out (Figs 43 A and 58), but they can be distinguished from the neutrophils by the bizarre and reddish appearance of their nuclei

b) Pappenheim's stain.

(May-Grünwald-Giemsa stain)

Necessary reagents:

i) *May-Grunwald solution,*

ii) *Giemsa stock solution*

1. Fixation of the air-dried preparation by immersion for 5 minutes in a dish of May-Grünwald solution. The solution can be used several times if protected from moisture (Use dry instruments and keep vessels closed). Rinse and dry the preparation

2 Stain as described under (a) 2

Result Differentiation between basophilic and acidophilic cytoplasm may be difficult, so that *monocytes* with indented nuclei are liable to be confused with the *juvenile forms of neutrophils* It is also difficult to judge whether the *neutrophils* show toxic granulation since all the neutrophils are granulated The basophils retain their granulation and the cells can be clearly recognized (Figs 43 B and 57) This stain is less suitable for fine differentiation than Giemsa but, on the other hand, it is good for the recognition of blood platelets

c) Wright's stain.

Necessary reagent *Wright's solution*

Fixation and staining are effected simultaneously by the addition of 20 drops of the staining solution to the air-dried preparation placed film side uppermost in a Petri dish Cover the dish and allow to stand 1—3 minutes according to the age and thickness of the preparation Then add dropwise an equal quantity of distilled water and leave for double the previous time (2—6 min.) Rinse and dry.

Result: Suitable for general differentiation and intermediate between Giemsa's and Pappenheim's stains

d) Wolfer's stain for proerythrocytes (reticulocytes).

Necessary reagent *0 5 % alcoholic solution of brilliant cresyl blue*

1 Preparation of slides Transfer the solution with a glass rod to one end of a microscope slide until approximately one third is covered Allow to dry, breathe lightly on the film of stain and spread evenly with a pad of cotton wool. Prepare a number of such slides and keep a reserve Stored in twos, film sides together and wrapped in paper, the slides remain usable for 2—3 weeks

2 Procedure: Prick the patient's finger and allow 2—3 large drops of blood to fall upon the prepared portion of one of the slides Cover with the prepared portion of a second slide so that only the coated parts of the slides are in contact. Taking the two slides by the uncoated ends, separate them and bring them together again several times in order to ensure thorough mixing of the blood with the stain Leave together for 3—5 minutes in a moist atmosphere (large covered Petri dish containing a wet swab). Then separate and allow to dry Alternatively, the blood on each of the slides may be collected with a spreader glass and spread out over the unused portion of the slide before being allowed to dry

3 Count the proerythrocytes to 1000 erythrocytes using an oil immersion objective and a counting eye-piece

Result The erythrocytes stain yellow to green, whereas the proerythrocytes contain the blue *substantia granulofilamentosa* (Fig 18 A)

e) Hirschfeld's stain for proerythrocytes (reticulocytes).

Necessary reagents

i) *Löffler's methylene blue,*

ii) *Methyl alcohol,*

iii) *Carbol gentian violet solution* as for bacteriological staining

1 First fixation, haemolysis and preliminary staining of the air-dried preparation. The film should be freshly prepared, in any case not more than 3 days old Allow 5 minutes' treatment with Löffler's methylene blue solution on the staining bridge The stain should be poured on rapidly but with care

2 Rinse carefully but thoroughly with distilled water and dry

3 Second fixation for 5 minutes in methyl alcohol Dry

4 Stain for 5—30 minutes in a dish of carbol gentian violet. Rinse well but with care in tap water and dry

- Count the cells using an oil *immersion objective* and a counting eye-piece.

[illegible]s the fixation is only very weak, the cedar oil may not be wiped [illegible]ff but should be removed by immersing the slide in xylene for [illegible] minutes Natural cedar oil must be used, since the synthetic [illegible]roduct dissolves the stain. The coloration fades after several [illegible]nonths.

[illegible]esult: The proerythrocytes may be distinguished from the ery[illegible]hrocytes by the presence in the cytoplasm of granulation and a [illegible]arying degree of reticulation The method is very convenient [illegible]when carrying out a series of examinations as it is not necessary [illegible]o stop after the removal of each blood sample to perform ad[illegible]itional operations, and staining can be delayed for as long as [illegible]hree days (Fig 18 B).

f) Peroxidase reaction of Graham-Knoll for general differentiation.

Necessary reagents: i) 10 % *alcoholic formaldehyde solution.* 10 parts of 40 % formaldehyde in 90 parts of 96 % alcohol

ii) *Peroxidase reagent* enough benzidine to cover the point of a knife is dissolved in 6 ml. 96 % alcohol, the solution diluted with 4 ml water and 0 02 ml hydrogen peroxide added In a closed vessel the reagent will keep for 5 days

iii) *Giemsa stock solution.*

The blood films should not be more than 1—2 days old.

1. Fixation of the air-dried preparation on the staining bridge *for exactly 30 seconds in alcoholic formaldehyde* Rinse with tap water and dry.
2. Allow the peroxidase reagent to act for 5 minutes by pouring over the preparation on the staining bridge. Rinse thoroughly with tap water and dry.
3. Stain as described under (a) 2, p. 27, but for twice as long. Rinse with tap water and dry.

Result A positive peroxidase reaction (yellowish green to brown granulation of the cytoplasm) is given by the eosinophils and neutrophils in all stages of development from promyelocytes onwards, and by a few of the basophils and some of the monocytes. Negative reactions are given by myeloblasts (neutrophiloblasts, eosinophiloblasts, *basophiloblasts*), *monoblasts* and *promonocytes*, as well as by some of the monocytes, the majority of the basophils and by all lymphocytes, plasma cells, megakaryocytes, platelets and erythrocytes (Figs 49—51) The neutrophils sometimes fail to react in certain parts of bone marrow films [illegible] the presence of fat. Portions of the film which are [illegible]

become so when the blood sample is kept [illegible] This true "peroxidase failure" can be recognized by the fact that, even in blood films, some of the mature, segmented *neutrophils* fail to react. A *peroxidase* failure in all the neutrophils has not so far been observed, a number of them always reacting positively. If the elements to be identified resemble neutrophilic promyelocytes and myelocytes in appearance, but all fail to give a peroxidase reaction, they are probably monocytes

g) Peroxidase reaction of Lepehne for erythroblasts.

Necessary reagents:

i) *Methyl alcohol,*

ii) *Lepehne's reagent* 2 ml of a 0 6 % solution of benzidine in 96 % alcohol are mixed, *shortly before use*, with 5 ml alcoholic perhydrol (0 5 ml 30 % perhydrol in 4 5 ml 70 % alcohol) The alcoholic benzidine solution has good keeping properties, but not the *mixed reagent*

iii) *Giemsa stock solution*

1. Ten minutes' fixation in methyl alcohol.
2. Fixation and reaction by 5 minutes' immersion in Lepehne's reagent. Rinse with *water and dry.*
3. Stain with Giemsa solution as described under (a) 2, p 27, but for twice as long

Result. The haemoglobin *is stained* brownish-yellow by the reaction Erythrocytes and *erythroblasts are strongly positive*, eosinophils slightly positive and proerythroblasts negative The reaction may also be carried out subsequent to panoptic staining. It enables erythroblasts to be distinguished from other cells with round nuclei (Fig. 31). A similar effect can be obtained with *staining method (f)* if the peroxidase reagent (Operation 2) is allowed to act for 30 *minutes instead of 5.*

h) Peroxidase reaction, modification I of Undritz for monocytes.

As staining method (f) except that, before commencing the procedure, the dried blood films are first stored singly side by side, unfixed and unstained, for 30—40 days in a protected place.

Result Similar to staining method (f), but the monocytes are *usually negative* (Figs. 52 A, 54). This modification enables the promonocytes and monocytes to be easily distinguished from the neutrophilic promyelocytes, myelocytes and metamyelocytes, which still give a strong positive reaction It is particularly suitable for bone marrow films provided that the presence of fat in the film examined does not interfere with the reaction

i) *Peroxidase reaction, modification II of Undritz for eosinophils.*

Necessary reagents.

i) *May-Grunwald solution,*

ii) *Giemsa stock solution,*

iii) *Peroxidase reagent*, see staining method (f)

1. Fixation with May-Grünwald solution as described under (b) 1, p. 27
2. *Staining with Giemsa solution* as described under (a) 2, p 27. except that 0 1 ml peroxidase reagent is added to every 10 ml water *before mixing with Giemsa's stain*

Result The *eosinophils give a strong positive reaction* (greenish-yellow) All other cells react negatively *except for a few basophils* which give *only a weak reaction* This method is suitable for the identification of eosinophils and their precursor forms, they show up very clearly, particularly in bone marrow preparations (Figs 52 B, 52 C and 182 H) It is also the best method for the differentiation of films prepared *from rabbit blood*

k) *Toluidine blue staining for basophils by the method of Undritz.*

Necessary reagent

Saturated solution of toluidine blue in methanol (approx 1 g toluidine blue in 100 ml methanol) The solution keeps indefinitely

1. Five minutes' *fixation* and *staining* of the air-dried film with the above reagent on the staining bridge
2. Rinse with water and dry

Table 5

DIFFERENTIATION OF BLOOD CELLS BY VARIOUS PEROXIDASE REACTIONS

o = negative (+) = some cells weakly positive

+ = weak positive reaction ++ = fairly strong positive reaction +++ = strong positive reaction

A frame round the finding signifies that it is characteristic of the species of cell

Species of cell (System)	Stages of development	Peroxidase reaction			
		For general differentiation (Graham-Knoll)	For erythroblasts (Lepehne)	Modication I for monocytes (Undritz)	Modification II for eosinophils (Undritz)
Erythrocytes	Proerythroblast	0	0	0	0
(Normocytes and megalocytes)	Erythroblast → erythrocyte	0	[+++]	0	0
Blood basophils	Promyelocyte I → segmented basophil	0 (+)	0 (+)	0 (+)	0 (+)
Eosinophils	Eosinophiloblast	0	0	0	0
	Promyelocyte I → segmented eosinophil	+++	++	+++	[+++]
Neutrophils	Neutrophiloblast (myeloblast)	0	0	0	0
	Promyelocyte I → segmented neutrophil	+++	0	+++	0
Monocytes	Monoblast → promonocyte	0	0	[0]	0
	Monocyte	0 → ++	0	[0]	0
Lymphocytes, plasma cells, megakaryocytes, platelets negative					
Non-haemopoietic cells of the bone marrow: negative					

Result: The granules of the basophils stain a brilliant reddish-violet (metachromasia) The remaining cells stain pale blue. The neurophilic granulation of neutrophils with toxic granulation and of the promyelocytes, promonocytes and blood platelets may show slight metachromatic coloration, but this is easily distinguished from that of the basophils *This method enables the blood and tissue basophils to be identified with certainty* (Figs 57 D and 192 H)

l) Silver impregnation for the stroma cells of the bone marrow by the method of Gömöri.

Necessary reagents.

i) *Methyl alcohol,*
ii) *0.5 % potassium permanganate solution,*
iii) *1 % potassium pyrosulphite solution,*
iv) *Ferrous ammonium sulphate* (2 % solution of iron alum, freshly prepared from the crystals as required)
v) *Gömöri's silver solution:* 2 ml. 10 % KOH solution are added to 10 ml 10 % $AgNO_3$ solution in a shaking cylinder. Concentrated NH_4OH is then added dropwise, shaking after each drop. After the precipitate has completely disappeared, 10 % silver nitrate solution is added dropwise with shaking until the precipitate which forms only disappears again with difficulty. Add an equal volume of distilled water In well closed bottles, the mixture will keep for 2 days
vi) *4 % solution of formol* (1 part formol 40 % in 9 parts water),
vii) *0 1 % solution of gold chloride,*
viii) *1 % solution of sodium thiosulphate*

1 Fixation of the air-dried preparation for 5 minutes in methyl alcohol Dry without rinsing
2 Immersion for 1—2 minutes in potassium permanganate solution Wash for 5 minutes with tap water.
3 Bleach for 1 minute in potassium pyrosulphite solution. Wash for 5 minutes with tap water
4 Immersion for 1 minute in ferrous ammonium sulphate solution Wash for 3 minutes with tap water, then twice with distilled water for 2 minutes each time
5. Immersion for 1 minute in Gomori's silver solution Rinse rapidly with distilled water (5 seconds)
6 Five minutes' treatment with formol Wash for 5 minutes with tap water.
7 Ten minutes' immersion in gold chloride solution Rinse with distilled water
8 Immersion in potassium pyrosulphite solution for 1 minute.
9 One minute in the sodium thiosulphate fixing solution Wash thoroughly with tap water Dry If the preparation is treated carefully, it is not necessary to embed it in Canada balsam

Result: Suitable for the recognition of stroma cells (fat cells), the only species of cell of the bones, bone marrow and blood which contains fibres of reticulin (Fig 199) *Films stained with* Giemsa's or Pappenheim's stain may afterwards be impregnated by this method

m) Staining of Heinz-Ehrlich bodies.

Necessary reagent·
0 5 % solution of Nile blue sulphate in absolute alcohol
Stain as described under (d) 1 and 2, p. 27, but using Nile blue sulphate solution instead of brilliant cresyl blue
Result. In certain types of poisoning, one or two small, dark blue bodies may be seen *near the edges* of the erythrocytes, which stain yellow to blue (Fig 20)

n) Fat staining with Sudan III by the method of Romeis.

Necessary reagents

i) *Hollborn's Sudan III.* The Sudan III is placed in an Erlenmeyer flask and 100 ml. 80 % alcohol added for each 1 g of stain. After fitting the flask with a cork carrying a reflux air condenser (a glass tube 1 2—1 5 metres long and 6—8 mm in internal diameter), the liquid is heated to boiling point on a water bath. The solution should be removed from the water bath shortly after boiling commences, longer boiling being of no advantage. The flask is then closed with a rubber bung, allowed to cool to room temperature, and afterwards placed for 1/2—1 hour in running water. The following day, the undissolved material is filtered off and the filtrate stored in a well-closed flask of alkali-free (Pyrex) glass This forms the stock solution which may be kept for years To prepare the staining solution, which contains only 40 % alcohol, a filtered quantity of a stock solution is mixed with an equal quantity of distilled water in a shaking cylinder, portions of 5 ml being added at a time. After each addition, the shaking cylinder is tilted to and fro ten times. The highly colloidal solution is transferred to a number of centrifuge glasses and centrifuged for 1/2 an hour It is then decanted carefully from any sediment which may be present and filtered If necessary, the filtrate can be used immediately for staining, otherwise it should be stored in a well-closed vessel Good colloidal solutions, if well sealed, will keep for 2—3 weeks but must always be filtered before use.

ii) *Mayer's or Ehrlich's haemalum*

1 Fixation in 4 % formol (1 part formol 40 % in 9 parts water) for 15 minutes Rinse thoroughly with tap water, dip three times in distilled water and allow to drain
2. Immerse while still moist in the Sudan III solution and leave for 6—8 hours at room temperature Wash thoroughly and rapidly with distilled water.
3 Nuclear staining by 15—20 minutes' treatment with haemalum Wash thoroughly with tap water for at least 1/2 an hour. Allow to drain and cover while still moist with glycerine-gelatine and a cover slip

Result: The reaction is strongly positive (orange) in the case of the neutrophils, and is even more pronounced in the case of the eosinophils. Only some of the monocytes react positively and the intensity varies. Very weak or negative reactions are given by the basophils, lymphocytes, megakaryocytes, platelets, plasma cells and erythrocytes In blood samples taken after meals, free fat droplets are often found between the cells and may be deposited on negatively reacting cells *The fat droplets (neutral fat) in the* vacuoles of the stroma cells of the bone marrow form large orange-red patches This method is suitable only for the detection of neutral fat When cedar oil or xylene is used, all the fat, together with the stain, is washed out

o) Fat staining with Sudan black B by the method of Lison.

Necessary reagents

i) *Sudan black B* 0 3 g Sudan black B is suspended in 100 ml *absolute alcohol, and the mixture* allowed to stand at room temperature with frequent shaking until complete solution is effected (1 to 2 days) Alternatively, the dye may first be finely ground in a mortar and the suspension afterwards heated
ii) *Buffer solution* A solution of 16 g pure phenol in 30 ml absolute alcohol is mixed with a *solution of 0 3 g $Na_2HPO_4 \cdot 12H_2O$* in 100 ml water.

Before use, 60 ml. of the Sudan black solution are mixed well with 40 ml of the buffer solution and filtered The mixture should be neutral or slightly alkaline The addition of the phenol makes the granulation more easily visible

1 Fixation of the air-dried preparation for 5 to 10 minutes in formalin vapour (the films are placed in a closed vessel the bottom of which is covered with 40 % formalin solution)
2. Immersion for 30 minutes in the Sudan black solution.
3 Thorough washing for several minutes in absolute or 70 % alcohol.
4 Second staining for 40 minutes with Giemsa solution as described under (a) 2, p 27.

A dirty grey appearance is due to the presence of neutral fat This can usually be avoided if the preparations are cleaned with xylene or examined under oil immersion. The cell lipoids, on the other hand, are not removed by this procedure, in contrast to Staining Method (n) *A positive reaction is given not only* by all the cells which give a positive Graham Knoll peroxidase reaction, Staining Method (f), but also by the lymphocytes, plasma cells, megakaryocytes and osteoclasts, though in these cases it is only weak and diffuse This can easily be verified by examining a preparation which has not received the second staining with Giemsa, e g a bone marrow film from a classical case of plasmocytoma. Consequently, the value of the reaction is reduced If a drop of distilled water is placed on the film and covered with a cover slip, as described by Storti and Perugini, it can then be examined under oil immersion without removing the neutral fat

p) Nuclear reaction of Feulgen.

Necessary reagents

i) *Methyl alcohol,*

ii) *Normal hydrochloric acid* 36 4 ml concentrated hydrochloric acid of S G. 1 19 are diluted to 1000 ml with distilled water

iii) *Fuchsine sulphurous acid* (Schiff's reagent) 1 g powdered fuchsine (parafuchsine) is dissolved in 200 ml boiling water in an Erlenmeyer flask by frequent shaking for 5 minutes After cooling to about 50° C, the solution is filtered into a flask with ground glass stopper, treated with 20 ml N-HCl and cooled to approximately 25° C under running water The solution is then bleached by treating it with 1 0 g anhydrous sodium bisulphite ($NaHSO_3$ *siccum pro analysi)* and allowing to stand at room temperature for at least 24 hours (If kept in the dark in a well-closed vessel, this reagent is stable for a long time) The final solution of fuchsine sulphurous acid, known as Schiff's reagent, has a pale yellow colour It should always contain a certain excess *of sulphurous acid to prevent decomposition,* indicated by a reddening of the solution The reagent should not be warmed

iv) *SO_2 water for rinsing* 200 ml tap water are mixed with

The bisulphite solution may be kept in stock in a well-closed vessel

1 Fixation of the blood film by 15 minutes' immersion in methyl alcohol or by passing through a flame
2 Hydrolysis immerse for 5 minutes in distilled water, then dip into cold normal hydrochloric acid and afterwards immerse for 4 minutes in normal hydrochloric acid warmed to exactly 60° and maintained at this temperature
3 Stop the hydrolysis by a brief immersion in cold, normal *hydrochloric acid and rinse with distilled water.*
4 Immerse while still wet in fuchsine sulphurous acid for 1—1 ½ hours. Wash by placing for 2 minutes in SO_2 water and repeating this operation twice more
5 Immerse for 5—10 minutes in tap water and dry. Examine under oil immersion

Result The nuclei and nuclear remnants of the blood cells (Howell-Jolly bodies, disintegrating nuclei) give a positive, reddish-violet coloration, all other cell constituents react negatively, i e remain unstained, including the cytoplasm of the megakaryocytes and the blood platelets (Figs 21 B and 21 C)

q) Prussian blue reaction of Grüneberg for the detection of siderocytes.

Necessary reagents

i) *Methyl alcohol,*

ii) *Solution of potassium ferrocyanide and hydrochloric acid* 1 g potassium ferrocyanide is dissolved in 1 ml. of official 25 % hydrochloric acid diluted with 99 ml water

iii) 0 25 % *aqueous solution of "Biebrich Scarlet"* or 0 1 % *aqueous solution of eosin.*

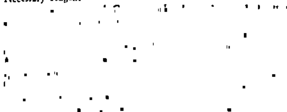
1 Fix the air-dried blood film for 5 minutes in methyl alcohol Dry
2 2—3 minutes' treatment with the solution of potassium ferrocyanide and hydrochloric acid on the staining bridge
3 Rinse with distilled water
4 2—3 minutes' counter-staining with "Biebrich Scarlet" or *eosin solution Rinse quickly and dry*

Result Small, blue, iron containing granules are seen in a number of the erythrocytes ("siderocytes") in certain diseases (Figs 19 D and 19 E) This method is also suitable for the detection of iron in monocytes

r) Thick drop method of Schilling.

Two fairly large drops of blood are transferred to a clean microscope slide so that they lie side by side, they are then spread out to a diameter of approximately 1 ½ cm with the aid of a needle and carefully dried in the air.

Necessary reagent



water and dried Examine under oil immersion

Result This method enables infrequently occurring elements, e g blood parasites and especially malarial parasites (Figs 242, 244 C, 244 D, 247 and 250) to be recognized more easily. It is also very convenient for making relative eosinophil counts

III. Forms for Recording the Results of Blood and Bone Marrow Examinations

a) Notes on the use of the blood picture forms

A single examination of the blood is often of decisive diagnostic value, but is seldom sufficient to enable the progress of the disease to be judged. To take full advantage of the possibilities offered by haematology, repeated blood examinations must be made at more or less frequent intervals, depending upon the nature of the disease The record form is therefore drawn up so as to enable the results of four different examinations to be entered side by side.

The use of the bone marrow examination forms is recommended for the differentiation of leukaemic blood pictures

The **colour of the plasma** can best be judged if a white background is placed behind the supernatant liquid 24 hours after commencing the sedimentation test.

The **leucocyte column**, which is found above the erythrocyte column in the sedimentation tube, is read in mm after 24 hours. 1 mm. on the Westergren tube corresponds to about 10,000 leucocytes per cu mm blood

Haemoglobin: a corrected reading of 100% or 100 units on the haemometer corresponds to 16.0 g. haemoglobin per 100 ml blood.

Colour coefficient = haemoglobin content of one erythrocyte expressed in micro-micrograms (10^{-12} g) (see p 37).

Proerythrocytes are the same as "reticulocytes".

The number of **normoblasts** is stated in terms of 100 leucocytes

Atypical cells. Only the total number is given The type to which they belong should be specified under "Remarks", e g "atypical lymphocytes" (characteristic of glandular fever) or "atypical myeloblasts, promyelocytes, etc". The description "atypical" is preferable to the prefix "para".

Granulation of the neutrophils with Giemsa staining should be denoted by 0, +, + +, + + + (see p. 27)

Under "**Anomalies**" enter any anomalies of the blood corpuscles

Under "**Remarks**" the presence of deformities, mitoses, etc should be noted

b) Notes on the use of the bone marrow forms

In bone marrow examinations actual differentiation of the cells is more valuable than cell counts, which may show more or less wide variations owing to contamination with blood The bone marrow sample may contain 30 to 99% blood Careful scrutiny of the preparation often reveals elements which pass unnoticed on superficial examination and which may prove of considerable diagnostic importance

The **simple form** is suitable for routine examination of uncomplicated cases and for leukaemic blood pictures for which the blood examination forms are inadequate Under the heading "Immature" in the case of the basophils, eosinophils and monocytes, are included all stages of development which do not normally enter the blood stream The lymphocytes, plasma cells and megakaryocytes are not subdivided

The **detailed form** is intended for special cases and for research work. If necessary, the cells with segmented nuclei may be subdivided into those with 2, 3 and more segments, or the form may be modified in other ways to suit special requirements.

In bone marrow examinations, percentage figures are given only for those leucocytes which are also found in the mature stage in the blood It is usual to count 500, the percentages being entered under **Group A** for comparison with the leucocyte counts of the blood picture Under **Group B**, the numbers of megakaryocytes and erythrocytes are entered, expressed as percentages of the leucocytes in Group A For all other species of cells, it is usually unnecessary to make a numerical determination, it is sufficient to indicate whether they are abundant, occasional or absent and whether they occur singly or in tissue formation, etc

Athrophagocytes are storage or phagocytic monocytes

Oxyphilic normoblasts I and **megaloblasts I** exhibit nuclear structure

Oxyphilic normoblasts II and **megaloblasts II** have no nuclear structure

Under the heading "deformed cells", the frequency of the deformity and the species of cell in which it occurs should be indicated

BLOOD PICTURE

Name ________________ Occupation ________________

Date of birth ________________ Diagnosis ________________

Date of examination					
Sedimentation Rate (Westergren)	1 hour				
	2 hours				
	24 hours				
Colour of plasma					
Leucocyte column in mm					
ERYTHROCYTES	Haemoglobin units				
	Haemoglobin grams %				
	Erythrocytes in cu mm				
	Colour coefficient				
	Diameter				
	Proerythrocytes pro mil				
	Polychromasia				
	Basophilic stippling				
	Anisocytosis				
	Poikilocytosis				
	Normoblasts per 100 leucocytes				
LEUCOCYTES	Leucocytes in cu mm				
	Basophils				
	Eosinophils				
	Neutrophiloblasts				
	Neutr promyelocytes				
	" myelocytes				
	Juvenile neutrophils				
	Staff neutrophils				
	Segmented neutrophils				
	Lymphocytes				
	Monocytes				
	Plasma cells				
	Atypical cells				
	Granulation of neutrophils				
	Doehle's bodies				
Blood platelets in cu.mm					
Anomalies					

Remarks and conclusions ________________

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BLOOD PICTURE

Name

Occupation

Date of birth

Diagnosis

Date of examination						
Sedimentation Rate (Westergren)	1 hour					
	2 hours					
	24 hours					
Colour of plasma						
Leucocyte column in mm						
ERYTHROCYTES	Haemoglobin units					
	grams %					
	Erythrocytes in cu mm					
	Colour coefficient					
	Diameter					
	Proerythrocytes pro mil					
	Polychromasia					
	Basophilic stippling					
	Anisocytosis					
	Poikilocytosis					
	Normoblasts per 100 leucocytes					
LEUCOCYTES	Leucocytes in cu mm					
	Basophils					
	Eosinophils					
	Neutrophiloblasts					
	Neutr promyelocytes					
	„ myelocytes					
	Juvenile neutrophils					
	Staff neutrophils					
	Segmented neutrophils					
	Lymphocytes					
	Monocytes					
	Plasma cells					
	Atypical cells					
	Granulation of neutrophils					
	Doehle's bodies					
Blood platelets in cu mm						
Anomalies						

Remarks and conclusions

BONE MARROW PICTURE

Simple Form

Name ________ Date of Birth ________

Diagnosis ________ Date of Examination ________

I. Haemopoietic cells

Group A (percentages)

		%
Blood basophils, immature		
„ „ mature		
Eosinophils, immature		
„ mature		
Neutrophiloblasts		
Neutr. promyelocytes		
„ semi-mature myelocytes		
„ mature myelocytes		
„ metamyelocytes		
Staff neutrophils		
Segmented neutrophils		
Lymphocytes		
Monocytes, immature		
„ mature		
„ phagocytic		
Plasma cells		

Group B (supplementary)

Megakaryocytes		
Pronormoblasts		
Macronormoblasts		
Basophilic normoblasts		
Polychromatic normoblasts		
Oxyphilic normoblasts		
Promegaloblasts		
Megaloblasts		

II. Non-haemopoietic cells

Tissue basophils	
Vascular cells	
Stroma cells	
Osteoblasts	
Osteoclasts	

III. Cells foreign to the bones and bone marrow

Tumour cells	
Skin elements	

Mitoses ________

Deformed cells ________

Macroscopic appearance of the marrow ________

Remarks and conclusions: ________

BONE MARROW PICTURE

Detailed Form

Name ______ Date of birth ______

Diagnosis ______ Date of examination ______

I. Haemopoietic cells

Group A (percentages)

1. Blood basophils

Basophiloblasts
B promyelocytes I
B promyelocytes II
B myelocytes
B metamyelocytes
Segmented basophils

2. Eosinophils

Eosinophiloblasts
E promyelocytes I
E promyelocytes II
E myelocytes
E metamyelocytes
Staff eosinophils
Segmented „

3 Neutrophils

Neutrophiloblasts
N promyelocytes I
N promyelocytes II
N semi-mature m cytes
N mature myelocytes
N metamyelocytes
Juvenile neutrophils
Staff neutrophils
Segmented „

4. Lymphocytes

Lymphoblasts
Prolymphocytes
Lymphocytes

5. Monocytes

Monoblasts
Promonocytes
Monocytes
Athrophagocytes

6. Plasma cells

Plasmoblasts
Proplasmocytes
Plasmocytes

Group B (supplementary)

7. Megakaryocyte-platelet system

Megakaryoblasts
Promegakaryocytes
Megakaryocytes

8. Normocytes

Pronormoblasts
Macronormoblasts
Basoph normoblasts
Polychrom „
Oxyphilic „ I
Oxyphilic „ II

9. Megalocytes

Promegaloblasts
Basoph megaloblasts
Polychrom „
Oxyphilic „ I
Oxyphilic „ II

II. Non-haemopoietic cells

Tissue basophils
Vascular cells
Stroma cells
Osteoblasts
Osteoclasts

III Cells foreign to the bones and bone marrow

Tumour cells
Skin elements

Mitoses of
Neutrophils
Normoblasts
Megaloblasts

Atypical forms of

Deformed cells

Macroscopic appearance ______

Remarks and conclusions ______

IV. Normal Values obtained on Blood and Bone Marrow Examination

1. Determinations of general, physical and chemical properties

a) Circulating blood volume

Infants	80—85 ml/kg
Children and adults	73—83 ml/kg

b) Specific weight

Whole blood, males	1 055—1 062
Whole blood, females	1 050—1 056
Plasma and serum	1 029—1 032

c) Hydrogen ion concentration

pH 7.25—7.40

d) Freezing point

— 0° 55 to — 0°.58 C

e) Viscosity (Hess viscometer)

Whole blood, males	4 74
Whole blood, females	4 40
Serum	1 6—2 0

f) Volumes (haematocrit method)

Packed cell volume

New-born	49—60 %
Children	32—44 %
Adults, male	39—52 %
Adults, female	35—48 %

Plasma

New-born	40—51 %
Children	56—68 %
Adults, male	48—61 %
Adults, female	52—65 %

g) Bleeding time (Duke's method)

1—3 minutes

h) Coagulation time

Schultz's hollow bead and capillary method

in capillary blood	4—9 minutes
in venous blood	up to 20 minutes

i) Prothrombin time (Quick's method)

12—13 seconds

k) Clot retraction time

Onset of retraction	after 1 hour
Marked retraction	after 18 hours

l) Sedimentation rate of erythrocytes (in low-lying districts)

	Westergren's method Normal maximum values after			Linzenmeier's method
	1 hour	2 hours	24 hours	
Males	5 mm	12 mm.	70 mm	350 minutes and longer
Females	10 mm	24 mm.	90 mm.	200 minutes and longer

N B Sedimentation rates vary in different individuals, and the normal values for many patients, particularly females, may be considerably below those given above Before it is possible to decide whether the sedimentation rate is pathological or not, it is necessary to know the minimum values exhibited by the patient when in a state of good health.

m) Takata-Ara reaction

Series of dilutions of serum with physiological saline 1 : 2 — 1 : 512

Normal conditions:

1 No marked flocculation after dilution 1 : 8
2 No initial flocculation after dilution 1 : 32

n) Weltmann's reaction
($CaCl_2$-heat coagulation threshold)

For preparation of the solution, use anhydrous $CaCl_2$ When the "Weltmann band" is normal, flocculation occurs on heating in the 1st tube *(0 05 %) or at higher concentrations up to the* 5th or 6th tube (0 03 or 0 025 %)

o) Wuhrmann-Wunderly nephelogram (extended Weltmann's reaction)

The Weltmann reaction is carried out using a larger series of dilutions and the opacity curve is determined on the tubes in which no flocculation occurs on heating

Normal opacity curves
From 0.03 % (5th tube) or 0.025 % (6th tube) to 0.006 % (11th tube) or 0.003 % (12th tube) $CaCl_2$ solution
Maximum at approx. 1000 opacity units

p) Wunderly-Wuhrmann's cadmium reaction (rapid method for testing the colloidal stability of serum):
0.4 ml. freshly centrifuged serum taken from a fasting patient and treated with 4 drops of 0.4 % cadmium sulphate solution normally gives no coagulation

2. Haemoglobin

a) Haemoglobin content

Stage of development	In corrected percentages or Sahli "units"	In grams per cent
New-born	100 — 150	16.0 — 24.0
Child	80 — 100	13.0 — 16.0
Adult	90 — 120	14.5 — 20.0

100 % or 100 units = 16.0 g in 100 ml blood (grams per cent). The exact determination in grams per cent is to be preferred to the conventional method of expression in percentage haemoglobin or haemoglobin units

b) Colour index

Calculation

$$\frac{\text{Haemoglobin \% (or units)}}{20 \times \text{erythrocytes (in millions per cu. mm)}}$$

Example

$$\frac{100}{20 \times 5} = 1.0$$

Normal values 0.9—1.1

c) Colour coefficient (haemoglobin content of a single erythrocyte in micro-micrograms [10^{-12} g])

Calculation

$$\frac{\text{Haemoglobin in grams \%} \times 10}{\text{Erythrocytes (in millions per cu. mm)}}$$

Example.

$$\frac{16 \times 10}{5} = 32$$

Normal values 28—36

d) Saturation index

Calculation

$$\frac{\text{Haemoglobin \% (or units)}}{\text{Packed cell volume / 100 ml}}$$

Example:

$$\frac{100}{45} = 2.22$$

Normal value, about 2.22

e) Haemoglobin concentration (in 100 ml erythrocytes)

Calculation

$$\frac{\text{Haemoglobin in grams \%} \times 100}{\text{Packed cell volume / 100 ml}}$$

Example.

$$\frac{16 \times 100}{45} = 35.5 \text{ g}$$

Normal values

New-born	approx. 45 g
Children	34—41 g
Adults	33—37 g

The colour coefficient and the haemoglobin concentration are to be preferred to the colour index and the saturation index since they are based on grams per cent and not on the conventional haemoglobin percentage or haemoglobin units

5. Chemical constituents

The figures refer to 100 ml. blood, unless otherwise stated.

$1\ \mu g = 1\ \gamma$

Constituent	Value	Unit
Acetone	1.2 — 2.6	mg
Alcohol	3 — 4	mg.
Bile acids	0 — 1	mg
Bilirubin	300 — 700	μg.
Bromine	1.4 — 2.7	mg
Bromine in serum	0.8 — 1.8	mg
Calcium	6	mg
Calcium in serum	9 — 11	mg
Calcium ions in serum	4 — 5	mg
Carbon dioxide in plasma (0°, 760 mm Hg):		
combined (alkali reserve)	50 — 65	ml
free	2.75	ml.
Carbon dioxide as bicarbonate	150 — 170	mg
Chlorine (as chloride)	285	mg.
Chlorine (as chloride) in serum	370	mg
Cholesterol, total	120 — 180	mg
Cholesterol in serum:		
esterified	approx. 140	mg
free	approx 60	mg
total	140 — 200	mg
Citric acid	1.7 — 2.7	mg
Copper	120	μg
Creatine and creatinine	5 — 7	mg
Creatine and creatinine in serum	3 — 7	mg
Fluorine	110	μg
Glucose	80 — 100	mg
Guanidine	250	μg.
Haemoglobin	16	g
Histamine in serum	2 — 7	μg
β-Hydroxybutyric acid	1.4	mg
Iodine	8 — 13	μg
Iron (in haemoglobin 0.34%)	54	mg
Iron in serum:		
females	105	μg.
males	125	μg.
Lactic acid	8 — 14	mg
Lecithin	175 — 300	mg
Lipase (stalagmometric)	0.005	U
Lipoids and fats	500 — 800	mg
Magnesium	2 — 4	mg
Methaemoglobin	300	mg
Neutral fat	360	mg
Oxalic acid in serum	2 — 5	mg
Phosphatase, alkaline, in serum in adults (Bodansky)	1.5 — 4.0	U
Phosphoric acid:		
Acid-soluble phosphorus	18 — 38	*mg*
Acid-soluble phosphorus in serum	2.5 — 5	mg
Lipoid phosphorus	7 — 14	mg
Lipoid phosphorus in serum	3 — 7	mg
Mineral phosphorus, adults	2 — 5	mg
Mineral phosphorus, children	4 — 5.5	mg
Total phosphorus	30 — 50	mg
Total phosphorus in serum	7 — 15	mg.
Potassium	200	mg
Potassium in serum	16 — 20	mg
Proteins in plasma	6.5 — 8	mg
by electrophoretic analysis:		
albumin 53.2% total protein	3.85 — 4.42	g
globulins 46.8% total protein	3.01 — 4.36	g
α_1-globulin 4.8% total protein	0.34 — 0.42	g
α_2-globulin 7.7% total protein	0.53 — 0.65	g
β_{1-2}-globulin 12.7% total protein	0.75 — 1.21	g
$\zeta = T$-globulin 4.0% total protein	0.29 — 0.34	g
φ = fibrinogen 2.8% total protein	0.19 — 0.24	g
γ_{1-2}-globulin 14.8% total protein	0.91 — 1.50	g
Purine bases	10	mg
Rest nitrogen	20 — 40	mg
Amino-acid nitrogen	5 — 8	*mg*
Ammonia (Conway)	4	μg
Ammonia (Folin)	80 — 110	μg
Ammonia (van Slyke)	50	μg
Creatine	4.5	mg
Creatinine	1	mg.
Indican	45	μg
Thiocyanogen	25 — 40	μg
Urea	10 — 20	mg
Uric acid	1 — 2	mg
Sodium	160 — 200	mg
Sodium in serum	280 — 350	mg
Sodium chloride	450 — 510	mg
Sodium chloride *in serum*	570 — 620	mg
Sulphur, inorganic	0.9 — 2.6	mg
Urea in serum	20 — 40	mg
Uric acid in serum	2 — 5	mg
Water	70 — 80	g
Xanthoprotein index in serum	1 — 2	mg

PART TWO

Systematic Survey of the Morphology of the Blood Corpuscles

In Part Two of the Atlas, detailed descriptions will be found of the various elements encountered in blood and bone marrow examinations. Since each type of element has its own specific origin and its own physiology and pathology, separate sections are devoted to each species of blood cell, to the non-haemopoietic elements of the bone marrow, to the adventitious cells and to the blood parasites. The description of each species of blood cell covers the whole range of normal development as well as the various abnormal forms caused by hereditary anomalies and by reactive or "primary" (leukaemic) disturbances in haemopoiesis. The reproduction of the blood cells is also discussed, including the deformities produced by abnormal karyokinesis.

Particular care has been taken to give detailed and accurate descriptions of the cells and to emphasize their distinguishing features. At the beginning of the section describing each species of cell, references will be found to the relevant illustrations, the numbers of those illustrations in which the cell is the main finding are printed in heavy type. Tables summarizing the characteristic features of the *stem cells of the blood corpuscles* (Tables 7, 8 and 9) and of *mature normal leucocytes* (Table 6) in panoptically stained preparations are given on pages 41 to 43.

Where the method of staining is not specified, one of the normal panoptic stains (Giemsa, Pappenheim or Wright, see p. 27) has been used; with these the acidophilic (oxyphilic, eosinophilic) constituents of the cells show up *red*, the basophilic *blue*, and the basophilic-metachromatic *violet*.

I. Table for the differentiation of mature leucocytes

Table 6 summarizes the most important properties of the mature forms of the 6 different species of leucocyte. The characteristics shown are sufficient to enable the various species to be identified, provided that no gross abnormalities are present. The **ability to recognize these elements forms the foundation upon which the whole structure of morphological haematology rests.**

A normal haemogram is shown on p. 38.

Table 6

DIFFERENTIATION OF MATURE NORMAL LEUCOCYTES IN PANOPTICALLY STAINED BLOOD FILMS

Cell species	Figure	Relative size and diameter	Shape of nucleus	Cytoplasm	
				Colour	Granulation
Pink cytoplasm: leucocytes derived from the bone marrow					
Basophil	43, 57, 58	medium, approx. 14 μ	segmented, polymorphous	pink	coarse, irregular, dense, **reddish-violet** (basophilic-metachromatic, dissolved by Giemsa)
Eosinophil	43, 63	medium, approx. 16 μ	usually bisegmented	pink	regular, vesicular, dense, **reddish-yellow** (eosinophilic)
Neutrophil	43, 74, 76	medium, approx. 14 μ	staff form or segmented, usually three segments	pink (uniform with Giemsa, mottled with Pappenheim)	none or fine and dispersed, **violet** (azurophilic)
Blue cytoplasm: leucocytes derived from the lymphatic-monocytic centres					
Monocyte	44, 109, 110	large 16 to 20 μ	lobed, indented or staff form	**pigeon blue** cloudy	very fine, dense, cloudlike formation, **violet** (azurophilic)
Lymphocyte	44, 132	small, approx. 12 μ	round or slightly indented	**clear blue**	none, or fine, sharply-defined single granules surrounded by haloes, **violet** (azurophilic)
Plasma cell	44, 145, 146	medium or large, 14 to 20 μ	round	**deep blue** masking red component	none vacuoles often present

II. Tables for the differentiation of the stem cells of the blood corpuscles

In Tables 7, 8 and 9 are summarized the characteristic features of the stem cells of the nine different species of blood corpuscles. With the aid of these tables it should be possible to identify all the various stem cells in whatever type of film they may be found.

The characteristics given apply only to normal stem cells. In pathological conditions, especially in leukaemias, many stem cells take on properties characteristic of others, so that the morphological differences become less obvious. Thus, the nuclei of the neutrophiloblasts, instead of being round, may become indented like those of normal monoblasts, or the nucleoli may coalesce to form a single nucleolus (nucleolar reduction), giving the cells the appearance of normal lymphoblasts. As the differences between the various stem cells are not very great, even under normal conditions, their differentiation in certain forms of stem-cell leukaemia constitutes one of the most difficult problems in morphological haematology.

Mistakes in the identification of cells are more likely to occur in the case of the pronormoblasts, which are readily confused with eosinophiloblasts, and the basophiloblasts, which may be mistaken for neutrophiloblasts or monoblasts.

The name **myeloblast** used by Naegeli is a collective term applied to basophiloblasts, eosinophiloblasts, neutrophiloblasts, monoblasts, plasmoblasts and megakaryoblasts. Ferrata's term **haemocytoblast** includes the proerythroblasts as well.

A normal myelogram is shown on p. 39.

Table 7

DIFFERENTIATION OF THE STEM CELLS OF THE BLOOD CORPUSCLES: DIFFERENCES IN CELL SIZE AND IN CYTOPLASM

Stem cell	Figure	Diameter (in μ)	Cytoplasm	
			Width	Basophilia
a) Pronormoblast	1, 22, 27, 30, 31	10–16	medium	intense
b) Promegaloblast	3, 25, 27, 31, 42	18–25	usually broad	intense
c) Basophiloblast	45, 55	12–15	narrow or medium	moderate or intense
d) Eosinophiloblast	45, 51	approx. 16	narrow	very intense; often achromatic spaces
e) Neutrophiloblast	42, 46, 51, 71	approx. 16	medium or broad	moderate
f) Monoblast	46, 51, 107, 122	15–22	medium	moderate
g) Lymphoblast	47, 131	12–14	narrow or medium	moderate
h) Plasmoblast	47, 143	approx. 16	medium	intense
i) Diploid megakaryoblast	47, 161	approx. 20	medium, often ragged	moderate

Table 8

DIFFERENTIATION OF THE STEM CELLS OF THE BLOOD CORPUSCLES: DIFFERENCES IN THE NUCLEI

Stem cell	Shape of nucleus	Chromatin network	
		Arrangement	Structure
a) Pronormoblast	round	radial tendency	distinct, fine, close mesh, abundant chromatin
b) Promegaloblast	round	radial	distinct, very fine, compact, short mesh
c) Basophiloblast	round or indented	long, skein-like threads	very indistinct, fine, long mesh
d) Eosinophiloblast	round	concentric	indistinct, very compact, short mesh, abundant chromatin
e) Neutrophiloblast	round	long, skein-like threads	distinct, fairly compact long mesh
f) Monoblast	round or indented	variable	indistinct, broad mesh
g) Lymphoblast	round	concentric	indistinct, f[illegible]
h) Plasmoblast	round	concentric	dense, [illegible]
i) Diploid megakaryoblast	round	not characteristic	indisti[illegible] scanty [illegible]

Table 9

DIFFERENTIATION OF THE STEM CELLS OF THE BLOOD CORPUSCLES: DIFFERENCES IN THE NUCLEOLI

Stem cell	Nucleoli				
	No	Size	Colour	Position in chromatin	Chromatin border
a) **Pronormoblast**	2—5	variable	blue	periphery covered	distinct, medium width
b) **Promegaloblast**	3—6	rather large	blue	periphery covered	distinct, medium width
c) **Basophiloblast**	2—6	medium	pale blue	variable	absent or very indistinct
d) **Eosinophiloblast**	2—4	medium, one very large	violet (masked)	covered	distinct, broad
e) **Neutrophiloblast**	2—6	medium	blue	usually uncovered	distinct, medium width
f) **Monoblast**	2—6	small	blue	variable	indistinct, rather broad
g) **Lymphoblast**	1—2	medium	blue	variable	distinct, medium width
h) **Plasmoblast**	3—6	medium	blue	usually covered	vague
i) **Diploid megakaryoblast**	2—6	small	pale blue	usually invisible	indistinct

III. The Erythrocytes

Figs 1 to 42, 48 A, 51 C, 231 A, 231 B, 236 L, 238 O, 238 P

1. CLASSIFICATION OF THE ERYTHROCYTES

Two different species or systems of erythrocytes are found in man the *normocytes* and the *megalocytes* The name "erythrocytes" or "red" blood corpuscles applies to cells of both systems and not merely to particular stages of development of one system,

"blast" in the same word is inadmissible The correct terms would be "erythroblast", "normoblast" and "megaloblast", but these have already been taken for the more mature stages of development

The normocytes and megalocytes resemble one another in

mitosis, the haemoglobin content increases very rapidly. In the early stages of development, the nuclei contain several nucleoli,

pathological conditions, the nuclei may become greatly reduced in size and form the so-called Howell-Jolly bodies The mature normocytes and megalocytes are non-nucleated In the pro-erythrocytes or reticulocytes, which are non-nucleated forms of either species that have not completed maturation, the cytoplasm contains *substantia granulofilamentosa*

One of the differences between the normocytes and megalocytes lies in the stage of ontogenesis at which they first appear The megalocytes make their appearance during the first month of embryonic development whereas the normocytes are not found until the third month The normocytes are considerably smaller than the megalocytes at the corresponding stage of development. Only the macronormoblasts (macroblasts) ever reach the size of megaloblasts. The nuclear structure of the normoblasts is coarser than that of the megaloblasts, and this enables the latter to be easily distinguished from macronormoblasts (Compare Fig 1 and Fig 2 cell b with Figs 3, 25 and 42) Moreover, macronormoblasts usually occur only during the early basophilic stages of development. On the other hand, the megaloblasts are large in all stages of development (from the promegaloblasts to the non-nucleated oxyphilic megalocytes). Macronormocytes (macrocytes) seldom reach the size of megalocytes and may be distinguished by their paler appearance Single, very large normocytes, which are occasionally encountered in healthy persons and, more frequently, in persons suffering from regenerative anaemias, are not megalocytes but mature, non-nucleated forms of what were originally binuclear normoblasts ("twinning deformities"), in other words, they are non-nucleated double cells (Fig 19 C) The chromosomes of the normoblasts (Fig 32) show a greater tendency to aggregation than those of the megaloblasts (Figs 37 to 41)

2. THE SYSTEM OF THE NORMOCYTES

Figs 1, 2, 5, 7 to 12, 15, to 23, 29, 30, 31 A, 32 to 36, 48 A, 51 C, 189, 231 B, 238 O, 238 P.

Normocytes and megalocytes together: Figs 13, 14, 26, 27.

STAGES OF DEVELOPMENT OF THE NORMOCYTES

a) Pronormoblast, Figs. 1 A left, 22 a, 27 top left, 30 left, 31 A. Tables 7, 8, 9, pages 42 and 43.

The pronormoblast is the stem cell of the system; it has a diameter of 10 to 16 μ. The nucleus is large and round and occupies almost the entire cell. It exhibits a fine, compact, chromatin network of regular, close mesh and containing several (2 to 5) blue nucleoli with a peripheral covering of chromatin The chromatin has not yet acquired the coarse, lumpy structure of the subsequent stages of development and there is only a suggestion of the radial arrangement characteristic of the normoblasts The cytoplasm has a clear, deep, and often somewhat uneven blue colour and, owing to the absence of haemoglobin, gives a negative response to the peroxidase reaction of Lepehne (Fig 31 A). The nucleus is sometimes surrounded by a pale halo. The pronormoblast is smaller than the promegaloblast and the nucleus has a coarser structure It differs from the stem cells of the leucocytes in the intense blue colour of the cytoplasm, the characteristic setting of the nucleoli and the close mesh of the chromatin network The pronormoblast is usually easy to identify, especially as it is frequently surrounded by more mature normoblasts. In some cases, large, strongly basophilic pronormoblasts may be very difficult to distinguish from eosinophiloblasts; generally speaking, however, the latter are larger, occur singly rather than in groups, and contain small, round achromatic patches and a violet, giant nucleolus (Figs 45 B, 45 C and 61 A).

b) Macronormoblast (macroblast), Figs. 1, 2 b (particularly large specimen), 51 C.

Diameter 18 to 22 μ. The exact position of this cell in the system is not yet certain It is possible that it represents an intermediate stage between the pronormoblast and the basophilic normoblast or that it is a particularly large form of pronormoblast. Normally, only isolated specimens are encountered in the bone marrow, but in many anaemias the numbers may be greatly increased (Fig 1) The structure of the nucleus and cytoplasm resembles either that of the pronormoblast or that of the basophilic normoblast It is frequently possible to distinguish several large blue nucleoli, the outlines of which glisten through the chromatin. Macronormoblasts are often confused with megaloblasts, but may be clearly distinguished by the coarser structure of the chromatin. Moreover, they are generally smaller than the megaloblasts (compare the macronormoblasts in Figs 1 and 51 C with the megaloblasts in Figs 3, 25, 27, 31 B and 42)

c) Basophilic normoblast, Figs 2 c, 22 b, 27 above, 29 left and 180

Diameter: 8 to 16 μ The round nucleus has a coarse, lumpy, radial structure and the nucleoli are usually no longer visible The dark blue cytoplasm still lacks any reddish coloration The Lepehne reaction is positive (Fig 31 A)

d) Polychromatic normoblast, Figs 1 C, 2 d, 22 c, 23 E, 26 below, 28 left, 29 right, 30 and 180.

Diameter: 8 to 12 μ. The round, coarse, lumpy nucleus is smaller than that of the preceding stage. The cytoplasm is no longer pure blue, but exhibits a reddish tinge, making it appear more violet, and finally a dirty greyish brown. It forms a wider band round the nucleus than in the basophilic stage.

e) Oxyphilic normoblast with nuclear structure, Figs 2 e, 22 d, 71 f, 73 A left, 100 A left, and as a frequent incidental finding in various other bone marrow illustrations

Diameter: 8—10 μ. The round nucleus is still very coarse and lumpy and has a well-marked structure. The cytoplasm is red with no trace of blue, and is broad, as in the subsequent stage.

f) Oxyphilic normoblast with structureless nucleus, Figs 2 f, 28, 116 B, 121 A below, and as a frequent incidental finding in various other bone marrow illustrations

Diameter: 7 to 9 μ. The small structureless nucleus (2 to 3 μ diameter) has become liquefied and homogeneous as the result of necrobiosis and is in the process of destruction; sometimes distortion has occurred and the uneven thickness gives a streaky appearance As in the case of the normocytes, the cytoplasm is red

The stages of development b to f are, as a rule, easy to recognise and confusion with other types of cell is scarcely possible. The coarse, lumpy, structure of the nucleus, the radial or "cartwheel" arrangement of the chromatin (b to e), the sharp demarcation of the cytoplasm without marginal thickening, the characteristic deep colour, and the frequency with which the cells are found lying together in groups or "nests", all enable these stages to be readily distinguished from leucocytes Confusion with disintegrating forms of the neutrophils, which are very occasionally found in the blood, is possible only in the case of stage f, where the nucleus has become liquefied and is therefore no longer characteristic of the erythroblasts (cf Fig 231 B with Fig 230 A) For confirmation, the peroxidase reaction of Lepehne may be employed, as all stages of development of the normocytes, with the exception of the pronormoblasts, give a positive result (Fig 31 A) The lymphocytes, plasma cells, and vascular endothelial cells, with which the normoblasts might be confused, all give a negative peroxidase reaction. Normoblasts may be distinguished from the corresponding stages of development of the megaloblasts by their smaller size.

g) Normocyte, Fig. 5, also present in most other illustrations

Diameter: 7 to 8.5 μ The normocyte is the mature form and is the final stage of development of the system It is non nucleated and has the appearance of a round, flat, biconcave disc. Even using panoptic stains, it is possible to differentiate cells containing polychromatic cytoplasm, or cytoplasm with basophilic stippling, from cells of a uniform red colour Special staining methods, such as staining with brilliant cresyl blue or the haemolytic method

(page 27, stains d and e), reveal a considerably larger number of cells containing basophilic material, the so-called *substantia granulofilamentosa* These cells are the **pronormocytes** or **reticulocytes** (Figs 18 and 20) They are younger than the cells which do not contain basophilic material and hence, in most cases, they are also larger The normocyte is not liable to be confused with any other type of cell.

Nucleoli are not visible in normoblast nuclei beyond the stage of polychromatic normoblast.

Mitosis of normoblasts (Fig 32)

The normoblasts reproduce by mitosis but only as long as the nucleus retains its structure Normally, therefore, cells capable of division do not enter the blood stream. Basophilic normoblasts in mitosis (Fig. 32 A) closely resemble lymphoblasts in mitosis (Fig 136), aggregation of the chromosomes is present to a marked degree in both, and the basophilic cytoplasm takes on a very mottled, granular appearance In practice, confusion between the two is very unlikely to occur, since, as a rule, mitosis of the normoblasts is seen only in the bone marrow, while mitosis of the lymphoblasts occurs in the lymph glands. In doubtful cases, e g. in lymphatic leukaemia with greatly increased proliferation and infiltration of the bone marrow, confirmation may be obtained by the Lepehne reaction—the normoblasts are positive (brown, Fig. 31 A), and the lymphoblasts negative (blue)

3. THE SYSTEM OF THE MEGALOCYTES

Figs. 3, 4, 6, 24, 25, 31 B, 37 to 42, 231 A, 236 L.

Megalocytes and normocytes together Figs 13, 14, 26, 27

STAGES OF DEVELOPMENT OF THE MEGALOCYTES

a) **Promegaloblast**, Figs 3, 25, 27 below, 31 B above, 37 to 40, 42, 179 A. Tables 7, 8 and 9, pp 42 and 43

Diameter 18 to 25 μ. The promegaloblast is the stem cell of the system. The round nucleus possesses an extremely fine, close-mesh chromatin network, the radial arrangement of which is clearly evident, even at this stage The nucleoli (3 to 6 in number) often run into one another and their outlines may be seen glistening through a covering of chromatin Surrounding the nucleus there may be a pale halo The cytoplasm has a deep blue colour and gives a negative Lepehne reaction as it does not yet contain any haemoglobin (Fig. 31 B) The normal form found in the embryo is smaller than that which occurs pathologically in pernicious anaemia (Fig 25) For the differentiation between promegaloblasts and macronormoblasts see pp 43 and 44

b) **Basophilic megaloblast**, Figs 3 above and left, 42, 24 A, 31 B centre, 39 B below, 40 C below, 42 top left

Diameter 16 to 20 μ The nucleus is round and the chromatin structure coarser than in the preceding stage The nucleoli are not always visible The cytoplasm is dark blue with no red component, but gives a positive Lepehne reaction (Fig. 31 B)

c) **Polychromatic megaloblast**, Figs 4 b, 24 B to 24 D, 31 B below, 37 B below

Diameter 12 to 16 μ The round nucleus possesses a coarse chromatin network arranged in "cartwheel" formation. No nucleoli are present The cytoplasm is polychromatic and forms a wide border

d) **Oxyphilic megaloblast with nuclear structure**, Fig 4 c.

Diameter: 10 to 12 μ The nucleus is round and comparatively small with a very dense structure The cytoplasm is wide and has a red colour with no blue component.

e) **Oxyphilic megaloblast with structureless nucleus**, Figs 3 below, 25 below, 26 above

Diameter 10 to 15 μ In the embryo, this stage tends to be somewhat larger than in patients suffering from pernicious anaemia (Fig 25) The nucleus is round, structureless and homogeneous, sometimes exhibiting a patchy appearance due to distortion, it is a typical necrobiotic nucleus The cytoplasm is red, as in the megalocyte

f) **Megalocyte**, Figs 4, 6, 13, 14, 23 C, 25

Diameter: 8 to 11 μ This is the mature cell, the final stage of development of the system. As in the case of the normocytes, special staining methods reveal the presence of *substantia granulofilamentosa* in some of these cells, which may therefore be characterized as promegalocytes

Nucleoli are not visible in the megaloblasts beyond the stage of the polychromatic megaloblasts.

Mitosis of megaloblasts, Figs 37 to 41 The megaloblasts reproduce by mitosis as long as the nuclear structure persists, the mature cells are no longer capable of division

4 THE ERYTHROCYTES UNDER ABNORMAL CONDITIONS

PERNICIOUS ANAEMIA (ADDISONIAN ANAEMIA, MEGALOCYTIC ANAEMIA, BIERMER'S DISEASE)

Under normal conditions, megalocytes are found only up to the third month of development of the embryo In certain pathological conditions, megalocytes reappear in adults. This occurs in all forms of pernicious anaemia—in the cryptogenic form, in anaemia of pregnancy, and in anaemia due to sprue or to diphyllobothrium From a practical point of view, only the megalocytes occurring in pathological conditions are of importance Apart from minor structural differences, the megalocytes found in the embryo are identical with those occurring in pernicious anaemia (Fig 25) Although megalocytes are the normal erythrocytes found in saurian blood, they are not adequate for the needs of the human adult with his higher phylogenetic development. Naegeli has termed them "morphological giants but functional dwarfs". Consequently, even in patients suffering from pernicious anaemia, the bone marrow still contains centres of normocyte formation and numerous normocytes are found in the blood (Fig 27)

HEREDITARY ANOMALIES OF THE NORMOCYTES

Microspherocytes (spherocytes, spherical cells), Fig 10.

These are normocytes which have adopted a spherical form *owing to reduced resistance*, and which therefore have a smaller diameter than usual. In consequence of their spherical form, the microspherocytes stain more intensely than ordinary normocytes. Microspherocytes are found in the haemolytic diseases and are particularly characteristic of *congenital haemolytic jaundice* (acholuric jaundice, familial haemolytic icterus), a dominant hereditary disease.

Elliptocytes (ovalocytes), Figs 7, 8, 30.

These are normocytes with an elliptical form They occur in *elliptocytosis* (ovalocytosis), another dominant hereditary anomaly. Individuals whose blood contains large numbers of elliptocytes are known as "complete carriers" (Fig 7), those with few *elliptocytes as "partial carriers"* (*Fig 8*) In complete carriers, more than 90 per cent of the erythrocytes may have the elliptical form Elliptocytosis is a true *primary* anomaly of the blood corpuscles, for the cytoplasm of the normocytes possesses a special structure which enables the cells to assume and retain the elliptical form. The earlier stages of development not found in the blood stream (normoblasts) remain round (Fig 30).

In pernicious anaemia, it may often happen that the megalocytes and normocytes have a slightly elliptical form, but this has no connection with elliptocytosis

The erythrocytes are *normally* elliptical in practically all primitive vertebrates with the *exception of the lamprey and the* tree frog, in both of which they are round. In mammals, elliptical normocytes are found only in the camel family. Even in these animals, the earlier stages of development are always round, the elliptical form appearing only in the circulating blood From the phylogenetic point of view, the elliptical form of the erythrocyte is older than the round form

Drepanocytes (sickle cells), Figs. 9, 15 A, 117 B

If blood is preserved in the liquid condition, the normocytes may, in certain *cases, become elongated and develop a curious* sickle-shaped appearance The precipitating cause is the increased concentration of carbon dioxide; in the presence of oxygen the cells again become round This deformation has no connection with the production of crenated forms which occurs in hypertonic media. Both types of deformation can occur side by side (see *Fig 9*). *The drepanocytes are usually darker in colour than* neighbouring normocytes which have not undergone deformation The tendency to sickle cell formation is the characteristic feature of a dominant hereditary anomaly, known as *drepanocytosis* (sickle cell anomaly) It is encountered only in negroes and mulattoes and is the only anomaly found in man which is restricted to a particular race A tendency to sickle cell formation is exhibited by 15 per cent of West African and American negroes Among white people, the anomaly has only been observed in populations which have lived for a long time in contact with negroes, and it may therefore be assumed that here, too, the cause of the anomaly *is* a predisposition inherited from the negroes. *In rare cases*, a haemolytic anaemia, *drepanocytic anaemia* or *sickle cell anaemia*, may develop in persons with a strong tendency to drepanocyte *formation. Like elliptocytosis, drepanocytosis is also a true* anomaly of the blood corpuscles, for these normocytes possess the inherent property of adopting the sickle form under certain conditions

Sickle cell formation in blood preserved in the liquid condition is a *normal* occurrence in many species of deer found in the Old and New Worlds In this case, it is an increased concentration of oxygen, not of carbon dioxide, which brings about drepanocytosis Under the influence of carbon dioxide the erythro*cytes become round* again

The *sickle cells* should not be confused with *half-moon* or *crescent bodies* (p 47) The half-moon bodies (Fig 11) are broader and paler than the drepanocytes and stain a pure red colour instead of yellowish-red They give a negative Lepehne reaction, whereas the drepanocytes react positively.

SECONDARY CHANGES IN ERYTHROCYTES; ARTEFACTS

Crenated forms (mulberry forms), Fig 12 A.

*These are erythrocytes with a serrated outline, and are pro*duced in blood films which have dried too slowly. Owing to evaporation, the surrounding plasma becomes hypertonic and the erythrocytes have time to shrink. This deformation is of a purely artificial nature, and can be shown by any erythrocyte It has no pathological significance

Microcytes (micronormocytes) Incidental findings on Figs 13, 17 A, 21 A

Some normocytes are inherently small and flat. As they are hypochromic (oligochromatic) they are paler than normal normocytes They are seen in many types of anaemia. It may often be very difficult to distinguish microcytes from fragments of fragile, hypochromic erythrocytes which have been torn during the preparation of the blood film.

***Macrocytes** (macronormocytes), Figs 17, 18, 88.*

These are normocytes with a larger diameter than usual Aetiologically, they do not form a homogeneous group The polychromatic normocytes in Fig 17 A, the normocytes with basophilic stippling in Fig 17 B, and the proerythrocytes in Fig 18 are all macrocytes Their size is merely a sign of immaturity In certain anaemias, e.g in many secondary anaemias, large *mature* normocytes are also encountered (Fig. 88) The large diameter of these normocytes is due to the fact that they contain less haemoglobin (oligochromasia, hypochromasia) and therefore spread out into a flatter form *(leptocytes, planocytes)*. Macro*normocytes differ from megalocytes in their low haemoglobin* content.

Target cells, Figs 15 A, 81 A

These normocytes have coloured centres and borders, separated by a pale ring, giving them a target-like appearance. As they are very thin, they are also known as *leptocytes* The presence of large numbers of target cells in the blood is pathognomonic of Cooley's anaemia *(Mediterranean anaemia, thalassanaemia)* but they also occur fairly often in diverse other types of anaemia. The target cells in Fig 15 A, for example, are from a case of *sickle cell anaemia Isolated target cells are also seen in the* blood of healthy persons

Anulocytes (ring cells, pessary cells), Figs 15 B, 17 A, 43, 50 A, 66, 87, 88, 90, 95, 96, 102 etc

These normocytes have a more or less large, pale area in the centre due to a lowered haemoglobin content They are particularly frequent in iron-deficiency anaemias Normal erythrocytes may undergo a similar deformation in thin portions of films prepared from the blood of healthy persons

Anulocytes with central acidophilic stippling, Figs 16, 102 A.

These are the same as the anulocytes just described, but have a fine red or reddish-violet granulation around the circumference of the central pale area. This granulation lacks the yellow component exhibited by other stained erythrocytes It is seen principally in preparations stained with Pappenheim stain The stippling is produced by local precipitation of the stain and is reminiscent of the Schüffner's dots seen in tertian malaria (Figs 245 C, 246 B), the latter, however, occupy the entire cell

Half moon bodies (crescent bodies), Fig 11

These are very large, pale, curved or elongated bodies, only found in thin portions of the film, generally where no other cells are present or partly covering other cells They are approximately uniform in size, and stain pure red with no yellow component The crescent bodies are particularly easy to see in preparations stained with Pappenheim stain, but are not visible in unstained preparations and give a negative Lepehne reaction for haemoglobin. They are artefacts formed from the stromata of particularly fragile erythrocytes which have lost their haemoglobin as the result of mechanical injury They are found only occasionally in healthy persons, but occur more frequently in anaemic patients Confusion with true sickle cells (drepanocytes, Fig 9), which are smaller and narrower and contain haemoglobin, is not likely.

Poikilocytes, Fig 14

Poikilocytes are small, irregularly shaped erythrocytes or fragments of erythrocytes (schizocytes) and are frequently pear-shaped They are formed mainly by mechanical division and deformation of particularly fragile erythrocytes and are encountered not only in pernicious anaemia, but in many other types of anaemia as well.

Burr cells, Fig 12 B

These are deformed erythrocytes, similar to poikilocytes, but having one or more pointed projections Their name derives from a somewhat remote resemblance to the clinging burrs of certain plants. They are liable to be confused with crenated forms (Fig 12 A) but generally occur singly whereas crenated forms are found in groups. Burr cells are seen in a number of diseases, especially in renal affections

Anisocytosis, Fig. 13

Anisocytosis is a term used to describe the presence of erythrocytes of several different sizes side by side It is a frequent finding in many types of anaemia and is particularly pronounced in pernicious anaemia where megalocytes and normocytes of [illegible]

[illegible]

duced deformation with certain secondary changes in the erythrocytes This is evidenced by the irregular distribution of these forms over the preparation and their dependence to some extent upon the staining method used Their practical significance is therefore limited Unless they occur regularly and frequently, they are of no diagnostic value in anaemia or other blood diseases

Polychromasia, Fig 17 A

Polychromatic erythrocytes are non-nucleated erythrocytes which still give a bluish coloration instead of pure red with the usual panoptic stains This is due to the fact that the maturation of the cytoplasm lags behind that of the nucleus, resulting in the abnormal persistence of the basophilic cytoplasm of the earlier nucleated stages Normal blood contains only isolated polychromatic normocytes

Basophilic stippling, Figs 17 B, 23, 28, 30

In the process of maturation of the erythrocytes, the basophilic substance generally remains diffused throughout the cytoplasm, becoming gradually paler until it finally disappears Under certain circumstances, however, it condenses to form small blue granules, a phenomenon known as basophilic stippling Even under normal conditions, some of the mature normocytes may show a very weak basophilic stippling, similar to that in Fig. 17 B, especially in women In pathological conditions, particularly in lead poisoning, the stippling may be coarser and the number of stippled erythrocytes much greater.

Proerythrocytosis (reticulocytosis), Figs 18, 20

The proerythrocytes have already been described as normal stages of development of the megalocytes and normocytes An increase in their numbers above the normal is pathological and is to be regarded as a sign of increased regeneration The number of proerythrocytes in the blood also shows a marked rise in patients treated for an aregenerative state of normopoiesis due to iron deficiency or to lack of the anti-pernicious-anaemia factor. This so-called pronormocyte or reticulocyte crisis is a valuable diagnostic aid and a criterion of therapeutic success

The presence in the blood of increased numbers of *polychromatic normocytes, normocytes with basophilic stippling* or *proerythrocytes* is always a symptom of increased normopoietic regeneration. In anaemias, an increase in these cells is, in itself, a good sign, since their absence indicates an aregenerative condition. However, a rise in erythrocyte and haemoglobin values can only take place when the causes of the anemia have been removed or when the rate of regeneration exceeds the loss

Siderocytes, Figs 19 D, 19 E; description on p 18

Heinz-Ehrlich bodies, Fig. 20, description on p. 18

Howell-Jolly bodies (nuclear globules), Figs. 4, 21 A, 21 B, 25 C, 33 B, 41 A, 173 C.

As the nucleus of the erythroblast matures, it gradually becomes smaller in size until it reaches a diameter of 2 to 3 μ. This is normally the critical size at which liquefaction takes place and the nucleus is completely absorbed These viscous nuclei stain deeply and are characteristic of the last nucleated stage of development of the normoblasts, "the oxyphilic normoblast with structureless nucleus" In various anaemias the normal destruction of the nucleus may be prevented so that instead of being suddenly absorbed, the nucleus continues to decrease in size until it reaches a diameter of about 1 μ These small nuclear remnants, known as Howell-Jolly bodies, are dark violet in colour, homogeneous

and have no central hof. They give a positive Feulgen reaction (Fig. 21 B) Sometimes two, and occasionally more, Howell-Jolly bodies are present in one erythrocyte (Fig 41 A left) During mitosis, aberrant chromosomes (Fig 41 A right) may unite to form Howell-Jolly bodies which are then seen lying in the cytoplasm beside the nucleus (Fig. 41 B) Normocytes containing Howell-Jolly bodies occur not only in anaemias, but are also a regular finding in cases of fibrosis of the spleen and in patients who have undergone splenectomy (Figs. 21 A, 21 B). Howell-Jolly bodies (Fig 173 C) should not be confused with blood platelets superimposed on erythrocytes The latter are surrounded by a narrow halo (Figs 173 A, 173 B) Open air-bubbles in the glass of faulty slides may take up stain and, if covered by erythrocytes, give the appearance of Howell-Jolly bodies (Fig 139 A)

Nuclear dust (chromatin dust), Fig. 19 A.

These tiny particles are smaller than Howell-Jolly bodies and usually only just visible They occur exclusively in non-nucleated erythrocytes and are composed of the remains of disintegrated nuclei Isolated particles of nuclear dust are sometimes found in healthy persons; larger quantities are associated with an increase in polychromatic erythrocytes and erythrocytes with basophilic stippling

Cabot's rings, Figs 19 B, 23 B

Cabot's rings are basophilic rings or loops found in erythrocytes Sometimes only fragments are present, as in Fig 23 B They occur only in severe anaemias, including untreated pernicious anaemia

Erythroconles.

These are azurophilic rods, up to 3 μ in length, found in erythrocytes in severe anaemias They were first described by Schilling.

Atypical nuclei and polyploidy of erythroblasts, Figs 19 C, 23 C, 32 C, 33 C, 34 to 36, 39 to 41, 238 O, 238 P. General descriptions on pp. 22 to 24

In many kinds of anaemia, *diploid* erythroblasts frequently contain *deformed nuclei*. Segmented nuclei, particularly those in which the segments form a rosette pattern, are often the result of interrupted, arrested mitosis (Fig 23 C)

The various types of abnormal cell development, including the most extreme forms, are well represented among the erythroblasts and are very easy to recognize Thus, examples of *all stages of polyploidy*, together with the corresponding mitoses, may be found among the megaloblasts (Figs 39—41); normoblasts in mitosis may contain giant chromosomes (Fig 35 C) and it is *possible to find highly polyploid mononuclear normoblasts with* enormous nucleoli (Figs 35 D, 36 E) and highly polyploid giant normocytes (Fig 36 F) In binuclear normoblasts (twinning deformities), *dissociation between proliferation and disintegration* in nuclei of the same age may occur (Figs 32 C, 33 C) See also p 16

Occurrence of erythroblasts in the blood, Figs 22 to 24, 189.

Nucleated erythrocytes do not normally enter the blood stream after birth. The presence of erythroblasts in the blood is therefore pathognomonic of regenerative erythropoiesis, as well as being characteristic of "toxic" and allergic diseases Thus, erythroblasts are released into the blood in many types of anaemia, but may also be found in the blood in other pathological conditions, including some in which the haemoglobin and erythrocyte values are normal or high In the presence of tumour metastases in the bone marrow and in leukaemic conditions, normoblasts may enter the blood stream as a result of a secondary disturbance, the so-called "crowding-out" effect; in erythraemia, the release of normoblasts into the blood stream is of "primary" origin. The presence of megaloblasts in the blood is a regular finding in untreated pernicious anaemia In haemolytic disease of the newborn due to rhesus incompatibility (Fig. 22), all stages of development of the normoblast, down to the most juvenile forms, may enter the blood stream in large numbers In Cooley's anaemia (Mediterranean anaemia), the appearance of normoblasts in the blood stream is due to constitutional factors and often occurs during crises or following infection

IV. The Leucocytes

Figs 42, 43 to 190, 227 to 231, 233 to 236, 238 N, 249, 254, 255

1. CLASSIFICATION OF THE LEUCOCYTES

Seven different species or systems of leucocytes are found in man *basophils with soluble granulation, eosinophils, neutrophils, monocytes, lymphocytes, plasma cells* and the *megakaryocyte-platelet system*

In the adult, the basophils, eosinophils, neutrophils, megakaryocytes and platelets are formed in the bone marrow, together with the normocytes The lymphocytes, monocytes and plasma cells are produced in the lymphatic or, more correctly, lymphatic-monocytic tissue in all parts of the body, including the bone marrow

The various species of leucocyte differ from one another in many respects These differences have already been mentioned in Part I and at the beginning of Part II (pp 41 to 43) and will be discussed in greater detail in the descriptions of the individual species All the leucocytes have, however, one feature in common the absence of haemoglobin, the pigment responsible for the intense colour of the erythrocytes They are therefore colourless, but sometimes appear white owing to refraction, and it is from this property that they derive the name of white blood corpuscles or leucocytes The inclusion of all the leucocytes in a single group distinct from the erythrocytes has certain practical advantages, for example when making absolute blood counts. However, the fact should not be lost sight of that the various species of blood corpuscle covered by the term "leucocyte" differ from one another at least as much as they do from the erythrocytes The only classification which reflects the actual biological conditions is the division of all the red and white blood corpuscles into nine different species

Normal absolute and relative leucocyte counts are given on p 38

2. THE SYSTEM OF BLOOD BASOPHILS

Basophils with soluble granulation or blood mast cells, often referred to simply as "basophils"

Figs 43 A, 43 B, 45 A, 49 A, 55 to 60, 62 F, 86 C, 181 A, 183 A, 189

STAGES OF DEVELOPMENT OF THE BLOOD BASOPHILS

a) Basophiloblast, Figs 45 A, 55 a, Tables 7, 8 and 9, pp 42 and 43

Diameter. 12 to 15 μ The basophiloblast is the stem cell of the system It has a round or indented nucleus which occupies almost the entire cell The pronounced metachromasia of the chromatin, which characterizes the blood basophil in all stages of development, is already evident, the nucleus staining redder than the nuclei of other species of blood corpuscle It has a fine, indistinct chromatin network and the individual fibres are long Two to six medium sized nucleoli are present. If the nucleoli are not covered by the chromatin, they appear pale blue. A striking feature which distinguishes this cell clearly from all other blast cells is that the chromatin breaks off sharply at the borders of the nucleoli instead of forming a thick ring round them The cytoplasm exhibits moderate basophilia and usually forms only a narrow band around the relatively large nucleus

Among other features, the comparatively small size of the basophiloblast gives it a close resemblance to the lymphoblast. The latter, however, contains more chromatin and usually has only one nucleolus The basophiloblast is distinguished from the neutrophiloblast by the blurred appearance of the chromatin network and the sharp demarcation between the chromatin and the small [illegible]

when the mature cells as well as the stem cells are present in increased numbers and when the stem cells are accompanied by the succeeding stage of development, the basophilic promyelocyte I The latter resembles the basophiloblast in appearance but already contains the specific granulation In normal bone marrow, the total number of basophils is so small that it is almost impossible *to identify the basophiloblasts*, which are extremely rare and very difficult to pick out.

b) Basophilic promyelocyte I, Figs 45 A, 55 b, 56 A, 56 C above

This cell resembles the basophiloblast in appearance but already contains a few specific metachromatic granules These are often very coarse

c) Basophilic promyelocyte II, Fig 56 B

The nucleoli are no longer visible and the cytoplasm, though still blue, contains many granules In this stage of development, the cell is approximately the same size as in stages **b** and **d** This is in contrast to the eosinophils and neutrophils which are particularly large in the promyelocyte II stage The illustration shows an unusually small specimen in a case of leukaemia

d) Basophilic myelocyte, Figs 56 C below, 56 D

The nucleus is still round but the cytoplasm is already pink.

e) Basophilic metamyelocyte.

The nucleus is indented but segmentation has not yet occurred

f) Basophils with segmented nuclei (segmented basophils), Figs 43 A, 43 B, 49 A, 57 to 59, Table 6, p 41

Diameter: 14 to 16 μ This represents the terminal stage of development of the system. The nucleus exhibits a varying degree of segmentation, sometimes has a clover-leaf form, and often appears as an indefinite lumpy mass The cytoplasm is pink.

Nucleoli. Even in the basophiloblast, the nucleoli can be recognised only with difficulty. In the more mature stages of development they are usually no longer visible, nor do they leave behind any traces in the chromatin in the form of the so-called "postnucleolar chromatin masses".

Mitosis and chromosomes. The mitosis of blood basophils can occasionally be seen in the bone marrow (Figs 60 A to 60 C) The mitotic angles are very obtuse and the chromosomes are thickened and lumped together

THE BASOPHILS IN HEREDITARY ANOMALIES OF THE BLOOD CORPUSCLES

In **Pelger-Huët's anomaly** all species of blood cell are affected, including the blood basophils In the heterozygotic form of the anomaly, the degree of segmentation of the basophils is less than usual, while in the homozygotic form in man, up to 75 % of the completely mature cells have round nuclei. **Alder's anomaly** (Fig 181 A) is characterized by the presence of azurophilic granules in the blood basophils In **May-Hegglin's anomaly** (Fig 183 A), some of the basophils contain Doehle's inclusion bodies

ATYPICAL FORMS OF BLOOD BASOPHILS

Owing to their rarity and their apparent unimportance from the practical point of view, little is known regarding atypical forms of blood basophils

In leukaemias, the granules of the blood basophils may vary greatly in number and size and in their affinity for stain.

Polyploidy. As in the case of all diploid blood cells, polyploid binuclear basophils are occasionally encountered, even under normal conditions In leukaemias, such forms are more frequently observed, and other cellular and nuclear anomalies may also be found (Fig 60 D)

EXCESSIVE PRODUCTION OF BASOPHILS

Both in the blood and at their site of formation in the bone marrow, the blood basophils are normally present only in small numbers They account for not more than 1 to 2 per cent of the leucocytes and are often absent altogether. In many allergic conditions, however, both the relative and the absolute basophil count may be increased, together with that of the eosinophils Large

numbers of basophils are a regular finding in polycythaemia vera. Basophils are also present in more or less large numbers in many cases of chronic myelogenous leukaemia and are always extremely numerous in **basophilic leukaemia** (Figs. 45 A, 55, 59). This very rare form of myelogenous leukaemia runs an acute or subacute course, with predominance either of basophilic promyelocytes (Fig 45 A) or of mature forms (Figs. 55, 59). A characteristic feature is the resistance of this form of leukaemia to therapy with arsenic or with X-rays

DESTRUCTION

The destruction of the basophils presents no special features

ARTEFACTS

Crushed basophils may be recognised by the granules lying scattered around the nuclear shadow.

DIFFERENTIATION OF THE BASOPHILS

For features which distinguish basophiloblasts from other blast cells, see Tables 7, 8 and 9, pp 42 and 43 In the later stages of development, granulation is always present and the cells can be readily identified by means of specific staining with toluidine blue (p 28) The basophils may also be distinguished from other cells with the help of Pappenheim staining Using Giemsa staining, the characteristic granulation is washed out. The basophils are most liable to be confused with neutrophils, from which they may be distinguished, however, by the fact that they stain a deeper pink and, in the case of segmented forms, by the curious shape of the nucleus Moreover, they generally give a negative peroxidase reaction in contrast to the neutrophils and eosinophils which are peroxidase-positive. In those blood basophils which give a positive reaction, the peroxidase is as stable as that present in the eosinophils, and a reaction can still be obtained in preparations which have been kept unfixed and unstained for as long as 5 years The peroxidases of the monocytes and neutrophils are much less stable, the former becoming inactive after one month and the latter after one year.

The blood basophils differ from the tissue basophils in the ease with which the granulation is washed out on fixation with methyl alcohol After Giemsa staining, the granules are therefore only partially visible, if at all, and may be replaced by vacuoles. The nuclei of the blood basophils are segmented, whereas those of mature tissue basophils are round (Figs 191, 192) The tissue basophils always give a negative peroxidase reaction.

In man, the tissue basophils do not normally enter the blood stream (an exception is mentioned on p 19) It is therefore possible to make a distinction between "blood basophils" and "tissue basophils". These terms cannot be used, however, in comparative haematology, for in certain animals both the blood basophils and the tissue basophils may pass into the circulation A more precise classification is that into "basophils with soluble granulation" and "basophils with insoluble granulation". The former include the blood basophils of man and the latter the tissue basophils.

3. THE SYSTEM OF THE EOSINOPHILS

Figs 43 C, 45 B, 45 C, 49 A, 52 B, 52 C, 61 to 70, 86 B, 181 B, 181 C, 182 H, 183 B, 189, 230 C.

STAGES OF DEVELOPMENT OF THE EOSINOPHILS

a) **Eosinophiloblast,** Figs. 45 B, 45 C, 61 A below; Tables 7, 8 and 9, pp 42 and 43

Diameter approx 16 μ. The stem cell of the system. The eosinophiloblast has a round nucleus which occupies almost the entire cell and is very rich in chromatin The dense, close-mesh structure is difficult to analyze but it can be seen that the chromatin fibres tend to be arranged concentrically The nucleus contains two to four nucleoli, a characteristic feature being that one is often very large, while the remainder are of medium size In consequence of the abundance of chromatin, even the giant nucleoli are always completely covered and therefore appear violet They can be recognized only by the comparatively broad and clearly visible chromatin border The cytoplasm is usually narrow, stains an intense blue, and may already exhibit small achromatic areas, the precursors of the specific granulation These achromatic dots may also be visible above the nucleus The larger vacuoles contain fat derived from stroma cells ruptured during marrow puncture (Fig 45 C above), fat is taken up very readily by the eosinophils at all stages of development. The eosinophiloblast is most likely to be confused with the pronormoblast (p 42)

b) **Eosinophilic promyelocyte I,** Figs *61* A above, *61* B below, *64* A above.

This cell is similar in appearance to the eosinophiloblast but the proportion of cytoplasm to nucleus is increased and azurophilic or eosinophilic granules are present in addition to numerous achromatic dots

c) **Eosinophilic promyelocyte II,** Figs *61* B above, *61* C, *62* D, *67*, *68*

Diameter. approx 20 μ This stage is considerably larger than the preceding one; the nucleus tends to be smaller but the cytoplasm has increased and is filled with granules, some of which are still azurophilic while others have already become definitely eosinophilic The cytoplasm itself is blue Nucleoli are very indistinct, often completely invisible.

d) **Eosinophilic myelocyte,** Figs 48 A, 52 C above, *62* E, *62* F bottom left, *67*, *68*.

Diameter: 16—18 μ. This cell is smaller than the preceding one and the cytoplasm stains *pink* The granules are sometimes still partly azurophilic, sometimes completely eosinophilic

e) **Eosinophilic metamyelocyte,** Fig *62* F bottom right.

The nucleus is indented.

f) Staff form of eosinophil (staff eosinophil), Fig 63 A above.

The distinction between eosinophilic metamyelocytes and staff forms is more or less an artificial one since, as a rule, the indented but not yet segmented nuclei of the eosinophils remain broad, except for a slight constriction at the point where the filament connecting the segments will later appear The structure remains relatively loose The "staff form" depicted in Fig 63 A is really still a metamyelocyte, but the two halves of the nucleus have been pulled apart causing the central portion to become narrowed.

g) Eosinophil with segmented nucleus (segmented eosinophil), Figs 43 C, 49 A, 52 B above, 63; Table 10, p 41.

Diameter. approx. 16 μ. The final stage of development of the system. The segments of the nucleus are connected by fine filaments which are usually very short In contrast to the neutrophils, the nuclei of which normally contain three segments, the nuclei of the eosinophils usually have only two segments, which are round or pouch-shaped. The cytoplasm is pink and filled with globular eosinophilic granules which are more or less regular in size In preparations which have been stained too strongly or rinsed in alkaline water, the eosinophils no longer appear red, but have more of a blue or dark violet colour

Granulation.

The granules of the eosinophils are first seen as small, round, achromatic spaces in the stem cells As a rule, some of them pass through an azurophilic stage, taking on various shades of blue and violet before finally becoming eosinophilic. The remaining granules are eosinophilic from the beginning In the early stages of development, the eosinophils sometimes contain peculiar vacuoles which are often of enormous size. These are filled with fat which is derived from stroma cells ruptured during marrow puncture and for which the eosinophils show a remarkable affinity (Figs. 45 C, 68).

Nucleoli.

Even when the eosinophils have reached the promyelocyte II stage, nucleoli can still be distinguished, and occasionally the characteristic giant nucleoli are also visible. Not infrequently, round or elongated postnucleolar chromatin masses are still present in the nuclear segments of the mature cells, as shown in the upper part of Fig. 63 B.

Mitosis and chromosomes.

Mitoses of eosinophils may occasionally be observed in bone marrow films (Fig. 69 A) The mitotic angles are obtuse and the chromosomes are thickened and aggregated The ability to proliferate is lost by the time the cell reaches the metamyelocyte stage, so that eosinophils capable of reproduction do not usually enter the blood stream.

Table 10

NUCLEAR PICTURE OF THE EOSINOPHILS

in normal persons and in three patients with a "shift to the right"

Each count included 50 to 100 eosinophils

	Figure	Diagnosis	Absolute leucocyte count	Eosinophils %	Nuclear forms of eosinophils in %				
					Unsegmented	Number of segments			
						2	3	4	5
a	—	Normal (average of 10 subjects)	7,300	3	4	**83**	13		
b	64 B	Hereditary hypersegmentation	7,000	4		10	**54**	32	4
c	64 C	Pernicious anaemia	6 500	9		**52**	28	18	2
d	65	Reactive shift in lymphogranuloma	over 100,000	90	4	25	**48**	18	5

Figures in heavy type denote the predominant form

THE EOSINOPHILS IN HEREDITARY ANOMALIES OF THE BLOOD CORPUSCLES

A moderate degree of hypersegmentation of the eosinophils (Fig 64 B) occurs as a hereditary, familial anomaly in which the majority of the nuclei contain three, sometimes even four or five, segments instead of the usual two (see Table 10, case b) In Central Europe this anomaly has so far been discovered only in one family, but several instances have been reported from Northern Europe (Sweden) Apparently only the heterozygotic form has so far been observed. This is not accompanied by clinical symptoms.

It is necessary to exclude the possibility of a purely reactive "shift to the right" which often occurs in the eosinophils in various diseases, especially in allergies. In such cases, it is possible for the nuclear picture to return to normal, but in the true anomaly no change occurs in the shift to the right.

A predominance of eosinophils with three nuclear segments is normal in the rabbit and many other animals

Eosinophils exhibiting various degrees of hypersegmentation of the nucleus are found in larger numbers than usual in patients suffering from pernicious anaemia (see Fig. 64 C and Table 10, case c) They may also occur as a reactive "shift to the right" in certain cases of lymphogranuloma accompanied by pronounced eosinophilia and leucocytosis (see Fig. 65 and Table 10, case d) as well as in so-called "tropical eosinophilia". In such cases, immature forms as young as the myelocyte may be found in the blood (Fig. 64 D)

In the heterozygotic form of **Pelger-Huët's anomaly** (see p. 54), the nuclei of the eosinophils have a particularly compact structure and it is not unusual to find mature eosinophils with round nuclei. In man and in many animals, it seems as though "pelgerization" of the eosinophils is a normal occurrence, which accounts for the predominance of bisegmented forms In the homozygotic form of Pelger's anomaly, the nuclei are always round, both in man (Fig 77A) and in rabbits In **Alder's anomaly,** the granules of the eosinophils are intensely azurophilic instead of eosinophilic and stain dark violet (Figs 181B, 181C above). That the cells in question are, in fact, eosinophils can be seen from the typical form of the nucleus and confirmed by means of the peroxidase reaction, modification II for eosinophils (p 28 and Fig 182H above) In **May-Hegglin's anomaly,** fragments of basophilic cytoplasm derived from earlier stages of development and similar to Doehle's inclusion bodies, are still present in the mature eosinophils (Fig 183B above)

ATYPICAL FORMS OF EOSINOPHILS

In reactive eosinophilias, the eosinophilic granulation in some of the cells may be largely replaced by azurophilic granulation (Fig 64D) analogous to the toxic granulation of the neutrophils In certain cases of chronic myelogenous leukaemia the eosinophils are remarkable for the fact that besides the specific granulation they also contain giant violet granules The latter resemble basophilic granules in appearance but are actually azurophilic and give only a feeble metachromatic reaction with toluidine blue. So-called microcells are particularly small juvenile forms of eosinophils (Fig 66), sometimes found in acute exacerbations of chronic myelogenous leukaemia. The juvenile forms of the basophils (Fig 56B) and the neutrophils (Figs 88, 89) may be similarly affected

Polyploidy. Polyploid eosinophils are occasionally found in normal persons in the form of binuclear cells (Figs 69, 70) Higher degrees of polyploidy and other types of nuclear anomaly may be observed in reactive states and in leukaemias (Fig 70).

EXCESSIVE PRODUCTION OF EOSINOPHILS

Eosinophils are nearly always to be found on examination of blood films, although normally they do not exceed 2 to 3 per cent of the total cell count. It is remarkable that a reactive increase in the number of eosinophils only occurs in association with certain phases of allergic disorders While it is possible that no eosinophils may be present in the peripheral blood in such cases, or that their numbers may be normal, they will always be found to be very numerous in the bone marrow or to have infiltrated in large numbers into the tissues at the site of the allergic reaction Thus, while eosinophilia always indicates an allergic condition, even the complete absence of eosinophils from the blood does not necessarily exclude such a disorder. In lymphogranuloma and in "tropical eosinophilia", a condition of still uncertain aetiology, the white cell count may exceed 100,000 per cu mm, over 90 per cent of the leucocytes being eosinophils In chronic myelogenous leukaemia, the eosinophils as well as the neutrophils and basophils are usually greatly increased in number True eosinophilic leukaemia is very rare (Figs 67, 68)

In conditions accompanied by very marked proliferation of the eosinophils (certain allergic diseases, eosinophilic leukaemia), very few eosinophiloblasts are found either in the blood or the bone marrow, a behaviour in contrast to that of all other blood cells It appears that, in the case of this cell, proliferation is maintained by the more mature elements, especially the promyelocytes. On the other hand, in conditions such as thrombocytopenia, where there is only a moderate increase in the number of eosinophils, the specific blast cells are more common in the bone marrow.

When making routine blood counts on normal persons, it is not unusual to find no eosinophils "Aneosinophilia" of this type is without significance In order to determine the number of eosinophils more exactly, the thick drop method of Schilling or the Fuchs-Rosenthal counting chamber must be employed These enable larger numbers of cells to be counted, and the error is thus reduced At the present time, these methods are much in use to test the functional activity of the adrenal cortex which secretes hormones causing a significant reduction in the number of eosinophils in the blood.

DESTRUCTION

The destruction of eosinophils proceeds in a similar manner to that of other cells It is to be noted, however, that the liquefied segments of the nucleus often coalesce to form a single round drop, which gives the cells a certain resemblance to myelocytes (see Fig 230C) Such cells have sometimes been found in histological sections and mistaken for eosinophilic myelocytes, which were assumed to have been produced directly in the tissues. In fact, however, they are necrobiotic eosinophils and their presence in the tissues is quite incidental

ARTEFACTS

Crushed eosinophils may be readily recognized by the granules lying in the vicinity of the nucleus

DIFFERENTIATION OF EOSINOPHILS

Normally, the eosinophils are easy to recognize owing to their characteristic granulation The distinctive features of the eosinophiloblast are shown in Tables 7 to 9, pp 42 and 43 All eosinophils except eosinophiloblasts give a very strongly positive peroxidase reaction Destruction of the peroxidase during fixation does not readily occur, and in unfixed preparations it retains its activity for at least five years This property of the eosinophils is made use of in modification II of the peroxidase reaction (staining method I, p 28) Using this method, only the eosinophils and a few easily recognizable basophils remain peroxidase positive. It thus enables the eosinophils to be clearly differentiated from all other cells (see pp 28, 29 and Figs 52B and 52C)

In the animal kingdom the eosinophils are not always peroxidase positive Negative reactions are given by the eosinophils of the urodela, the felines, the African and Indian rhinoceros and the South African rock rabbit In the hyena, not only are the eosinophils peroxidase negative, but they do not even give an eosinophilic staining reaction as their granules are chromophobic. The pseudo-eosinophils found in rabbits, elephants and many other animals, are really neutrophils in which the acidophilic substance of the cytoplasm is more highly granulated than in man.

4. THE SYSTEM OF THE NEUTROPHILS

Figs 42, 43, 44 A, 46 D, 48—54, 71 to 108, 181 to 183, 185 to 187 A, 188 B, 189, 227 to 231, 233 B, 235, 236 I and 236 K.

STAGES OF DEVELOPMENT OF THE NEUTROPHILS

a) Neutrophiloblast, Figs. 42 top right, 46 D, 51 A above, 71 a, 88, 89, 95 top right, 100, 189, 231 C, Tables 7, 8 and 9, pp 42 and 43

Diameter approx 16 μ. The stem cell of the system. The neutrophiloblast is the most common species of myeloblast, even when the blood picture is normal, and in pathological conditions this is still more true For this reason, the term myeloblast is usually employed as synonymous with neutrophiloblast. The nucleus is round but smaller in relation to the cytoplasm than in the case of the basophiloblasts and eosinophiloblasts. It contains a moderate amount of chromatin arranged in a clearly defined, convolute network, the individual fibres being long and of medium strength The nucleus contains two to six (usually three or four) nucleoli As these are often uncovered, their clear, blue colour can be plainly seen The nucleoli are of medium size and surrounded by a well-marked border of chromatin. The cytoplasm tends to be broad and stains a vivid, clear blue colour As in the case of all blast cells, no granulation is present. The peroxidase reaction is negative (the so-called "myeloblasts" or "paramyeloblasts" which give a positive peroxidase reaction are cells in a later stage of development, usually promyelocytes)

b) Neutrophilic promyelocyte I, Figs 53, 71 b, 88, 90, 98 B, 98 C, 104, 105

This cell resembles the neutrophiloblast in appearance, but already contains a few azurophilic granules, in the neighbourhood of which the peroxidase reaction is positive. The nucleoli are often clearly visible This cell is liable to be confused with the promonocyte, but the latter is usually peroxidase negative and the nucleus frequently indented.

c) Neutrophilic promyelocyte II (immature neutrophilic myelocyte), Figs 46 D, 53, 54, 69 A 71 c, 87, 91, 92, 93, 94 A, 97, 99 D, 99 E, 101, 106 A, 235, 236 I, 236 K.

Diameter· 22—25 μ All neutrophils pass through this giant stage which corresponds to that of the eosinophilic promyelocyte II The nucleus is round, and nucleoli may still be visible The cytoplasm is filled with azurophilic progranulation and is peroxidase positive throughout. This cell is unlikely to be confused with any other

d) Semi mature neutrophilic myelocyte, Figs 71 d, 83 A, 84, 97

Diameter 18—20 μ The nucleus is round and smaller than in the preceding stage, the cytoplasm is blue but also contains a pink component There is less azurophilic progranulation than in the preceding stage

e) Mature neutrophilic myelocyte, Figs 53, 71 e, 72 a, 84, 99 F

Diameter 16—18 μ The size of the nucleus has further decreased and the nucleoli are no longer visible The cytoplasm is generally pink with no blue component and no azurophilic granulation.

f) Neutrophilic metamyelocyte, Figs 72 b, 84, 85 B, 85 C, 86 A, 95, 96

The nucleus is slightly indented (Arneth's W-cell) The structure of the chromatin is closer than in the preceding stage, but still relatively loose. No nucleoli are visible The cytoplasm is pink.

g) Juvenile neutrophil, Figs. 65 bottom left, 72 c, 73, 75 C, 84, 95, 97, 102 B, 102 C, 106 B.

The nucleus is more deeply indented than in the metamyelocyte The chromatin network is still loose. When making neutrophil counts, the metamyelocytes and juvenile forms are usually grouped together and described either simply as metamyelocytes or as juvenile forms

h) Staff form of neutrophil (staff neutrophil), Figs. 44 A below, 52 B centre, 72 d, 73, 74, 75 A, 75 B, 83 B, 84, 85 A, 95, 110 F below, 183 B below, 187 A, 188 B, 189

The nucleus is deeply indented (Arneth's T-cell), slender, and continuous throughout its length, i.e not yet segmented. The cytoplasm is pink Even when the indentation of the nucleus extends to the opposite side at only one point, the cell can no longer be considered a staff form, but a segmented neutrophil

i) Neutrophil with segmented nucleus (segmented neutrophil), Figs 43, 46 D, 49 to 52, 54, 72 e to 74, 76 to 82, 84, 86 C, 89, 94 B, 95, 96, 103, 109 C, 112 G, 142 A, 181 to 183, 189, 233 B, Table 6, p 41

This is the final stage of development. The chromatin is compact and somewhat lumpy, and small post-nucleolar chromatin masses are sometimes present. The nucleus is elongated and almost completely severed at one or more points It thus consists of two, three, four or more segments, which generally remain connected either by very short, narrow bridges, or by fairly long threads of chromatin.

Granulation.

The mature neutrophils usually have a very fine, dense, acidophilic (eosinophilic) granulation which shows up with Pappenheim but not with Giemsa stain. It is so delicate that it is only visible on close observation. Azurophilic ("toxic") granulation, if present at all, is usually very sparse

Nucleoli.

The nucleoli of the neutrophils disappear, at the latest, in the myelocyte stage, but may leave behind a "post-nucleolar chromatin mass" In leukaemias, giant nucleoli are sometimes observed. These arise through the fusion of several nucleoli of normal size, and their formation is therefore accompanied by a reduction in the number of nucleoli (see Figs 88 and 90) Thus, in many acute myelogenous leukaemias, the neutrophiloblasts and neutrophilic promyelocytes may contain only one or two nucleoli instead of the usual three or four, giving them a resemblance to lymphoblasts In these cases, therefore, other criteria, such as the peroxidase reaction (see Fig 90 B), must be used to distinguish between these two species of cell

Mitosis and chromosomes.

The neutrophils are diploid cells. They may continue capable of proliferation up to the stage of the metamyelocyte Only the more mature staff forms and neutrophils with segmented nuclei enter the blood stream The chromosomes of the neutrophils are usually of moderate thickness and clearly visible, and the mitotic angles are obtuse (Figs 98 to 100, 104 A, 104 B).

Table 11

NUCLEAR PICTURE OF THE NEUTROPHILS

he percentages for normal people quoted from Arneth are averages of 10 values, our own figures are averages f 20 (both sexes). The remaining figures relate to preparations obtained from patients with hypersegmentation ("shift to the right") and with reactive nuclear shift ("shift to the left") (Figs. 75, 80, 81 and 82).

	Figure	Diagnosis	Nuclear forms of neutrophils in %										
			Unsegmented		Number of segments								
			Juveniles	Staff forms	2	3	4	5	6	7	8	9	10
a	—	Normal (Arneth)		5	35	**41**	17	2					
		Normal (own figures)		5	20	**50**	22	3					
b	80	Hereditary hypersegmentation			5	28	**40**	22	4	1			
c	81 A	Hypersegmentation in pernicious anaemia of pregnancy	6		8	20	**26**	18	15	4	3		
d	81 B	Hypersegmentation in cryptogenic pernicious anaemia		1	6	15	**32**	22	15	5	4		
e	82	Hypersegmentation in septic conditions (rare)	3		13	22	**30**	16	11	1	1	2	1
f	75 A	Nuclear shift in pulmonary tuberculosis		**33**	32	26	7	2					
g	75 B and 75 C	Nuclear shift in pneumonia	**27**	21	17	25	7	3					

Figures in heavy type denote the predominant form.

DIFFERENTIATION BETWEEN SEGMENTED NEUTROPHILS AND STAFF FORMS

From the practical point of view, it is particularly important to be able to distinguish between these two kinds of neutrophil As Arneth and Schilling have shown, an increase in the number of staff forms occurs in a great variety of diseases and their abundance gives an indication of the extent to which the disease is active Confusion between segmented neutrophils and staff forms will be avoided if microscope slides and not cover slips are used to prepare the smears, when cover slips are used, the nuclei are likely to be distorted by the pressure. The examination of slide preparations should be confined to thin portions of the film where the cells are usually undamaged and spread out to the best advantage Normally, out of every 100 neutrophils, five are staff forms, the majority have three nuclear segments and more than five segments is very rare (see Table 11, letter a) *Normal neutrophils thus have a moderate degree of segmentation of the nucleus*

THE NEUTROPHILS IN HEREDITARY ANOMALIES OF THE BLOOD CORPUSCLES

Pelger-Huët's nuclear anomaly of the leucocytes (Pelger's anomaly) (Figs 77, 78, 97, 101 A) This is a dominant hereditary anomaly and has so far been observed in man and in rabbits In man, the heterozygotic form of Pelger's anomaly is not uncommon and in Switzerland, for example, more than 40 families have been found to be affected On the other hand, the homozygotic form remained unknown in man until 1951, and only the one case has so far been reported In rabbits, it proved possible to breed homozygotic individuals as far back as 1942

In Pelger's anomaly, the neutrophils are the principal cells exhibiting abnormality If the neutrophils are "pelgerized", all the other blood cells are to some extent affected too In the **heterozygotic form** (Figs. 77 B, 78 B, Table 12, p 55, cases b and e), most of the neutrophils have unsegmented indented nuclei (in man 54 %, in the rabbit 88 %) The impression is therefore created of a regenerative nuclear shift, for which reason the anomaly has also been called the "pseudo-regenerative white blood picture" *The remaining neutrophils have only two segments,* apart from a few isolated cells which may have three The nuclear shift is thus of quite a different nature from that seen in infections, in which neutrophils exhibiting a higher number of segments are always present (Table 11, cases f and g) The nuclei and segments are remarkably short and thick, rich in chromatin, and often contain large post-nucleolar chromatin masses

In the **homozygotic manifestation** of Pelger's anomaly (Figs 77 A, 78 A; Table 12, examples **a** and **d**) the chromatin is so dense *that the majority of the neutrophils (up to 94 % in man and up to 100 % in rabbits)* retain their round form even when completely mature. The basophilic chromatin, though not segmented, is broken up into large, irregular fragments which are surrounded by the mobile, oxyphilic chromatin. In man, about 6 % of the neutrophils have indented nuclei and a bisegmented form may occasionally be seen. Although the percentage of neutrophils with round nuclei is higher in rabbits than in man, the anomaly is the *same in both cases, the difference being of a secondary nature* determined by the species, even in rabbits exhibiting the heterozygotic manifestation, the shift to the left is more pronounced than in man.

In **partial carriers of Pelger cells** (Figs 77 C, 78 C, Table 12, examples **e** and **f**), a form of the anomaly which has not yet found a satisfactory genetic interpretation, Pelger neutrophils are found side by side with normal neutrophils in a constant ratio. Partial carriers are found frequently in rabbits, but in man partial carriers with more than 1 % Pelger neutrophils have so far been reported in only two families. In one family, only one member, a woman, had Pelger neutrophils the ratio being 20 % Pelger cells to 80 % normal neutrophils. Neither the parents nor any of the other 15 close relatives had Pelger cells. In the other family (unpublished case of Prof. Stodtmeister, Pforzheim, Germany), a father and son both exhibited Pelger cells, the proportion being 80 % Pelger neutrophils and 20 % normal neutrophils.

Persons exhibiting the heterozygotic and homozygotic manifestations of Pelger's anomaly are complete carriers, as all their leucocytes are "pelgerized".

Leukaemic patients may also exhibit Pelger's anomaly (Fig. 97).

In many species of animals, the leucocytes are normally of the heterozygotic or homozygotic Pelger type.

Pseudo-Pelger-cells (Figs 79 A, 79 B, 79 C) are ordinary neutrophils the nuclei of which have taken on forms very similar to those of *heterozygotic Pelger cells* under the influence of regenerative stimuli. They were first observed by Heilmeyer and Rohr in cases of leukaemia, and later were also found in severe infections, especially in enteritis. In infections of this type, more than half the neutrophils have a normal appearance and some cells containing three or four segments are always present. Even in the remaining neutrophils, the chromatin structure is usually less lumpy than in true Pelger cells. The presence of Doehle's inclusion bodies, toxic granulation and vacuoles is characteristic of such acute infections. No hereditary factor is involved, and on recovery the pseudo-Pelger-cells disappear.

Fig. 79 D shows neutrophils with round nuclei in heterozygotic Pelger rabbits after colchicine injection. They imitate true homozygotic Pelger nuclei, but are larger and the chromatin shows no fragmentation. These cells, too, disappear again rapidly from the circulation.

Table 12

NUCLEAR PICTURE OF THE NEUTROPHILS IN PELGER'S ANOMALY

The corresponding preparations are shown in Figs. 77 and 78.

	Figure	Species	Diagnosis	Nuclear forms of neutrophils in %					
				Unsegmented		Number of segments			
				round	indented	2	3	4	5
a	77 A	Man	Homozygotic Pelger	**94**	**6**				
b	77 B	Man	Heterozygotic Pelger, complete carrier		**54**	**45**	**1**		
c	77 C	Man	Partial carrier, approx 20 % Pelger cells		7 + **8**	16 + **11**	32	22	4
d	78 A	Rabbit	Homozygotic Pelger	**100**					
e	78 B	Rabbit	Heterozygotic Pelger, complete carrier		**88**	**12**			
f	78 C	Rabbit	Partial carrier 7 % Pelger cells		10 + **3**	26 + **3**	33 + **1**	20	4

Figures in ordinary type: normal neutrophils
Figures in heavy type: Pelger neutrophils

Hereditary hypersegmentation of the nucleus in neutrophils is another dominant anomaly which occurs both in man and in rabbits The majority of the neutrophils have nuclei containing four segments instead of the usual three, while occasionally six or more segments may be present (see Table 11, case b, p. 54, and Fig. 80) So far, only the heterozygotic form of this anomaly has been observed.

In **Alder's anomaly** of leucocyte granulation (Figs 181, 182), the neutrophils, too, contain very numerous azurophilic granules Such a degree of granulation is scarely ever attained in cases of reactive, so-called "toxic", granulation With Pappenheim staining, the granules even obscure the nucleus, which stains less intensely. Partial carriers of the anomaly have also been seen, their blood always containing a small proportion of neutrophils with profuse azurophilic granulation.

In **May-Hegglin's anomaly,** the presence of Doehle's inclusion bodies in the cytoplasm is also a constant finding in the neutrophils (Fig 183 B).

ATYPICAL FORMS OF NEUTROPHILS

Microforms. In acute myelogenous leukaemia and in acute exacerbations of chronic myelogenous leukaemia, greatly increased proliferation of the neutrophils occurs, but often only microforms are produced. The reduction in size is effected mainly at the expense of the cytoplasm, the cells having the appearance of practically denuded nuclei (Figs 88, 89). Apparently, the amount of [illegible]

Forms with atypical nuclei. In many types of leukaemia, the nuclei of the neutrophiloblasts, the neutrophilic promyelocytes and the myelocytes are indented instead of round These cells are then known as paramyeloblasts or paramyelocytes, or, more correctly, as "atypical" myeloblasts or myelocytes

Polyploidy. Polyploid neutrophils are not rare, and even in normal blood it is possible to find large, mature neutrophils containing two segmented nuclei (Figs 102 D, 103) Although, owing to the presence of the two nuclei, these cells contain a larger [illegible]

in leukaemias, where all stages of development are affected, including even neutrophiloblasts (Figs 105, 106) The atypical mitoses and endomitoses which give rise to these monster cells may also frequently be seen (Fig 104)

Toxic bone marrow and toxic granulation. During infective diseases and in cases of poisoning, the azurophilic progranulation of the promyelocytes in the bone marrow may be abnormally profuse ("toxic marrow", Fig 84) This azurophilic granulation persists as "toxic granulation" up to the most mature stages of development When recording the results of blood examinations, the intensity of the toxic granulation is generally denoted by one, two or three plus signs (see pp 16, 27 and Fig 76) It shows up most clearly with Giemsa staining

[illegible]

as granules These Auer rods are present even at the promyelocyte I stage, the very young forms containing only a single crystal in place of granulation (Fig 90 A) A positive peroxidase reaction is given only in the vicinity of the Auer rod (Fig 90 B). In promyelocytes II, Auer rods may be present alone (Fig 87 A above), or together with granulation (Fig 87 B), while there are always some promyelocytes which contain only granulation and no rods (Fig. 87 A below). Where a large number of Auer rods are present in a promyelocyte, they may have a dense, felt-like appearance (Fig 92).

Doehle's inclusion bodies. In pathological conditions, not only the azurophilic progranulation but also portions of the juvenile basophilic cytoplasm may persist into the mature stages These basophilic fragments, known as Doehle's inclusion bodies, are found in many neutrophils in infective diseases, especially in scarlet fever and pneumonia (Figs 75 B, 75 C)

Vacuoles are sometimes present in the neutrophils in pathological states (Figs 85 A, 105) In juvenile forms, the vacuoles may be arranged in a rosette pattern (Figs 104 C, 106 A). They appear to contain a protein-like substance

EXCESSIVE PRODUCTION AND DIMINISHED PRODUCTION OF NEUTROPHILS

In man, the neutrophils constitute the most abundant species of leucocyte and in normal blood, they account for 45 to 70 per cent of the white blood cells In the majority of leucocytoses caused by infective disease or poisoning, the number of neutrophils is greatly increased. On the other hand, their numbers are normal or only slightly increased in leucocytoses due to lymphatic glandular fever and infectious lymphocytosis Myelogenous leukaemias, particularly the acute and sub-acute forms, are usually characterized by very pronounced neutrocytosis. The so-called "acute and sub-acute myelogenous leukaemias", the most important of which are the "myeloblastic leukaemias", include those with **predominance of neutrophiloblasts** (Figs. 88, 100, 231 C), **neutrophilic promyelocytes I** (Fig 90) and **neutrophilic promyelocytes II** (Figs 87, 91, 92) Such leukaemias are said to be atypical when, as is often the case, they are accompanied by nuclear malformations (Figs 93, 94) In chronic leukaemias, the disease is not generally confined to a single species of cell, but affects simultaneously the neutrophils, basophils and eosinophils (Figs 95, 96, 189) Similarly, during the acute stages of chronic myelogenous leukaemia, not only neutrophiloblasts but also basophiloblasts and eosinophiloblasts appear in large numbers (Figs. 56 B, 66) In certain leukaemias in which only the basophils, eosinophils, lymphocytes or plasma cells are affected, small numbers of neutrophilic promyelocytes and even of neutrophiloblasts may be found in the blood. The expulsion of these cells into the blood stream is apparently a secondary process similar to the so-called "crowding out" of normoblasts which often occurs in leukaemias. For further details regarding leukaemias, see pp 14 and 15

A reduction in the absolute number of neutrophils in the blood is known as **neutropenia.** This condition may be without importance except when the neutrophil count reaches very low values. Severe neutropenia accompanied by clinical symptoms is most frequently found in agranulocytosis ("aneutrocytosis" or "malignant neutropenia") and in the so-called splenopathic depression of the bone marrow In chronic neutropenia accompanied by *clinical symptoms, splenopathic depression of the bone marrow* should always be suspected, since the life of the patient may

often be saved by splenectomy, even when no cells are found in the bone marrow. The haematological findings need not always be accompanied by enlargement of the spleen, but when present this symptom greatly facilitates the diagnosis As a rule, agranulocytosis runs an acute course, whereas splenopathic depression of the bone marrow is a chronic affection.

Shift to the left (Arneth) or regenerative nuclear shift (Schilling). Pathological stimuli often lead to an increase in the number of staff forms in the blood, more immature stages of development, such as juvenile forms, myelocytes and promyelocytes may also appear in the peripheral circulation Thus, in acute infective diseases the number of unsegmented neutrophils may increase by 50 per cent or more (see p 54, Table 11, case g, and Figs 75 B and 75 C) In chronic infections such as pulmonary tuberculosis, however, it is usually only the staff forms which show marked hyperplasia (see Table 11, case f, and Fig 75 A) Apparently, the presence of large numbers of staff forms does not necessarily indicate a shift to the left, but may be due to the pathological stimuli inhibiting the development of the maturing nucleus Whatever may be the causes of the shift, the determination of the percentage of unsegmented forms has proved of great practical value

In acute infective diseases, especially in pneumonia and in severe intestinal infections, neutrophilic promyelocytes may also appear in the blood. In myelogenous leukaemias, the shift to the left may extend even to the neutrophiloblasts, particularly in acute forms and in the acute phases of chronic forms

Shift to the right. When the majority of the neutrophils contain nuclei with more than three segments, the condition is termed a shift to the right (Arneth) It frequently occurs in diseases of the liver, and especially in pernicious anaemia (see Table 11, cases e and d, p 54 and Fig 81) The precursors of these hypersegmented forms are the so-called neutrophilic giant metamyelocytes of the bone marrow (Figs 85 B, 86 A), in fact, however, these cells are not true metamyelocytes but myelocytes with prematurely indented nuclei

A shift to the right may also occur very occasionally in septic conditions, instead of the usual shift to the left (see Table 11, case e, and Fig 82) In some of the nuclei the segments are arranged in a rosette pattern, giving the impression of an arrested mitosis

DESTRUCTION

Destruction of the neutrophils follows the same course as that of all other blood corpuscles (pp 10, 11, 17, 18, Plate 40) It should be noted that disintegrating neutrophils, the nuclei of which have condensed to a single drop (Figs 227 D, 230 A), are liable to be confused with normoblasts containing nuclei in a physiological stage of destruction (Fig 231 B) The peculiar disintegrating forms of neutrophils found in acute disseminated lupus erythematodes (Figs 185, 186) are described on p 18

ARTEFACTS

At one time, much discussion centred around the granulated "Ferrata cells" found in cases of myelogenous leukaemia and in bone marrow films Some haematologists maintained that they were very young stages or parallel histoid stages of development of normal leucocytes, while others held that they were more mature cells which had become artifically deformed. All the evidence is in favour of the latter view (Figs 235, 236 I, 236 K)

Crushed segmented neutrophils (Fig 233 B), may be distinguished from crushed lymphocytes (Fig 234), by the fact that their nuclei form several small shadows instead of a single large one

DIFFERENTIATION OF THE NEUTROPHILS

The distinctive features of the neutrophiloblast are shown in Tables 7 to 9, pp 42 and 43 See also Figs 45 to 47

Peroxidase reaction. Neutrophiloblasts always give a negative peroxidase reaction. Normally, all the remaining stages of development of the neutrophil, from the promyelocyte I to the segmented neutrophil, are peroxidase positive In unfixed preparations, neutrophils lose their ability to give the peroxidase reaction in about eight months The peroxidase reaction, especially modification I for monocytes (pp 28, 29), enables neutrophilic promyelocytes to be distinguished clearly from promonocytes and monocytes This is of considerable diagnostic importance, particularly as atypical promyelocytes may closely resemble monocytes in appearance· they may then be recognized by their positive peroxidase reaction For examples of the practical application of the reaction see Figs 53, 54, 93 If the procedure is suitably carried out, it may be used for differentiation even in cases where some of the neutrophils have lost their peroxidase (see Fig 94 and p 28)

Differentiation between immature neutrophils (juveniles and staff forms) and monocytes with deeply indented nuclei. Generally speaking, monocytes with deeply indented nuclei are seldom encountered When they do occur, they may be mistaken for juvenile forms of neutrophils and thus lead to a false diagnosis of a shift to the left. The two forms may be distinguished by the fact that the cytoplasm of the monocytes stains blue while that of the neutrophils stains pink (see Figs 44 A, 83 B) Moreover, the nucleoli of the monocytes contain less chromatin and the network is looser. Good staining is essential for differentiation, Giemsa stain giving the best results With Pappenheim stain, over-staining of the preparation may sometimes render differentiation difficult.

5 THE SYSTEM OF THE MONOCYTES

Figures 44 A above, 44 B above, 46 E, 46 F, 49 D, 50, 51 B, 52 A, 54, 83, 107 to 130, 143, 147, 179, 180, 181 A, 181 D above, 182 H below, 183 C below, 186 D, 186 E, 187 A above, 188 A, 190, 213, 214, 227 A, 227 E, 228 G, 228 H, 229, 233 C, 249, 254, 255

STAGES OF DEVELOPMENT OF THE MONOCYTES

a) Monoblast, Figs 46 E above, 46 F above, 51 B above, 107 A, 107 B, 108 D above, 122 A above, 122 B, 124, 129 A above, 129 C, 190, 213, 214, Tables 7, 8 and 9, pp 42 and 43

Diameter 16 to 22 μ The stem cell of the system. The nucleus is round, sometimes slightly indented, and not very rich in chromatin. The chromatin network is very fine and indistinct, it has a skein-like appearance and the individual fibres are long As a rule, several (2—6) small nucleoli are visible Their position

the chromatin is variable and the chromatin border tends to be road and indistinct. The amount of cytoplasm varies in relation the size of the nucleus but usually forms a moderately wide ' . of a fairly intense, cloudy blue colour Like all blast cells, monoblast is ungranulated and the peroxidase reaction is

..

) **Promonocyte**, Figs. 46E below, 46F below, 51B below, 54, C, 108, 111, 112, 114, 115, 116A, 118A, 122, 123, 125, 127, 28C, 129B, 130, 190, 213, 214

The size of the promonocyte varies within the same limits as of the monoblast. The nucleus is usually indented but ccasionally still round. This stage embraces several generations 'ch may be distinguished by the degree of indentation of the The nucleoli are usually no longer visible, and the cyto- is more abundant than in the preceding stage. In contrast the neutrophilic promyelocyte, the promonocyte is generally eroxidase negative (see Fig. 51B below) The cytoplasm is lways blue and comparatively pale. It shows a distinct azuro- hilic progranulation with Pappenheim's stain, but with Giemsa's tain and the peroxidase reaction the granulation is only weakly visible, if at all.

) **Monocyte**, Figs 44A above, 44B above, 49D below, 50, 52A, 4, 83, 108E below, 109, 110, 111D, 112F, 112G, 113 to 122, 123 right, 126, 129B below, 143 centre, 147, 179, 180, 181A above, 181D above, 182H below, 183C below, 186D, 186E, 187A above, 188A, 233C, 249, 255; Table 6, p 41

This is the final stage of the system. The nucleus is occasionally round, but is generally more or less deeply indented with two or even three lobes In the latter case, it acquires the appearance of a capital E. Frequently, the lobes lie one above the other, particularly if the cell is not very thinly spread out The nucleus contains relatively little chromatin and has a remarkably loose structure which is very characteristic. Neither nucleoli nor post-nucleolar chromatin masses are present The cytoplasm has a dull, pigeon-blue colour and exhibits dense clouds of very fine, irregularly distributed, azurophilic granules which are best seen with Pappenheim staining

Mitosis and chromosomes.

The monocytes are normally diploid cells In most cases, their ability to proliferate probably ends at the promonocyte stage, but the results of blood-culture experiments suggest that some mature monocytes may still be capable of mitosis In leukaemic monocytosis, it is by no means rare to find dividing monocytes in the blood, and they are also an occasional finding in agranulocytosis and other diseases accompanied by intense monocytic reactions The chromosomes of the monocytes are thick with obtuse angles and have a greater tendency to aggregation than those of the neutrophils (Figs 124, 125, 128A, 128B)

POLYMORPHISM OF THE MONOCYTES

The monocyte is the most polymorphous of all the blood cells In addition to the normal round form found in the haemopoietic organs and blood, it also occurs, either as a fixed or as a migratory cell, in the interstitial tissue of the organs, where its shape is decided by the nature of the surroundings Thus, the Kupffer cells of the liver, which satisfy all the criteria for monocytes, owe their star-like appearance to the long cytoplasmic processes forced out by the peculiar column-like arrangement of the liver cells The appearance of the monocytes may also be modified by functional activity. The monocytes are, in fact, the most antixenic of all the blood cells and act as the scavengers of the organism; they destroy and consume corpuscular elements (phagocytosis) and store up colloids (athrocytosis), for which reason they are also known as athrophagocytes The great variety of forms assumed by the monocyte is largely due to the diversity of substances taken up and to the varying quantities consumed. As a rule, the monocytes found in the blood in healthy persons are comparatively uniform in appearance, while those in the organs are polymorphous as they are much more actively engaged in phagocytosis and athrocytosis The more foreign matter taken up by the monocytes, the less motile they become. In man, phagocytic and athrocytic monocytes are found in the peripheral blood only in the embryo and in persons suffering from certain diseases In many species of animal, however, athrophagocytes are a regular finding in the blood, even in adults.

Ehrlich termed the monocytes "large mononuclear and transitional forms". Owing to their great variation in appearance, the monocytes, particularly those found in the tissues, have been described under many different names Thus, all the following cells appear to be monocytes: histiocytes, histioleucocytes, histiomonocytes, "polymorphous histiogenic migratory cells" (Weidenreich), histiocytic endothelial or reticuloendothelial cells (Aschoff and Kiyono), certain alveolar epithelial cells, Kupffer's stellate cells of the liver, splenocytes, clasmatocytes (Ranvier) "primitive migratory cells" (Saxer), macrophages or macrophagocytes (Metchnikoff), "specific endothelial cells" (Simson), "leucocytoid cells of vascular adventitia" (Marchand), polyblasts (Maximow), "rhagiocrin cells" (Renaut), "lymphoconjunctive cells" (Dominici), "phagocytic reticulum cells" of the bone marrow (Rohr), "pulp cells" of the spleen (Moeschlin), the "plasma cells" of plasma cell pneumonia, epithelioid cells, Langhans' giant cells, "foreign-body giant cells", the "sympathogonias" found in the blood in certain "sympathogoniomas", and Gaucher cells

PHAGOCYTIC AND ATHROCYTIC MONOCYTES

In blood and bone marrow films, all types of monocytes may be found, from the non-phagocytic to the athrophagocytic forms Even the promonocytes are capable of phagocytosis and storage (see Figs 111A to 111C, 112E, 114, 115, 118A) A promonocyte which contains remains of ingested material and is in the process of mitosis is shown in Fig 125A. The peculiar necrobiotic forms found in acute disseminated lupus erythematosus are also consumed by phagocytic monocytes (Figs 186D, 186E). It is very probable that the positive peroxidase reaction given by some monocytes is due to the remains of peroxidase-positive material consumed during phagocytosis (Figs 112E, 113, 179B) The vacuoles occasionally observed in monocytes in healthy persons may also indicate that phagocytosis has taken place This is certainly true of the vacuoles in septic diseases, particularly in meningococcal sepsis (Fig 114) and in endocarditis lenta (Fig 113) In films prepared from cutaneous eruptions in patients suffering from meningococcal sepsis, all stages of phagocytosis and vacuole formation may be observed While some monocytes contain intact bacteria, in others the meningococci have been partly digested and vacuoles have begun to form in the surrounding cytoplasm, in the final stage, vacuoles are present which contain only liquid material The formation of vacuoles at points where

phagocytosis of bacteria has taken place, can also be followed directly *in vitro*. In Leishmaniasis (Kala azar) the *Leishmania* are almost invariably found only in the monocytes of the internal organs, but occasionally a few are also present in the monocytes of the blood (Fig. 249). In the Oroya fever stage of Carrión's disease, the infecting parasites *(Bartonella bacilliformis)* are also found in the monocytes (Fig. 254). A combination of phagocytosis and athrocytosis is seen in the monocytes of the bone marrow in malaria, especially in the estivo-autumnal variety. The parasites are first engulfed and digested by the monocytes, and the indigestible pigment then stored (Figs. 115 A, 115 B).

In man, monocytes of high phagocytic activity remain fixed in the tissues and become converted into senile, degenerate forms. These cells attain a great size, up to 60 μ in diameter, the cytoplasm being distended with the remains of ingested cells (Fig. 117 B) or with stored material (Figs. 118 to 121). The nucleus has usually become round and is reduced to a small, dry structure, a phenomenon which can also be observed occasionally in the phagocytic monocytes in the blood (Fig. 112 F). In this form, athrophagocytic monocytes seem capable of continuing their existence for a very long time.

Special forms of athrophagocytic monocytes include the following.

1. **"Embryo macrophages".** These normally circulate in the blood of the human embryo and usually contain a large variety of cells in the process of destruction. How unselective the phagocytosis of stimulated monocytes may sometimes be is illustrated by Fig. 116 A.

2. **Athrophagocytic monocytes of the bone marrow** ("phagocytic reticulum cells" of Rohr). These are a regular finding even in normal bone marrow. They are responsible for the removal of the large quantities of waste material continuously formed in this highly active tissue (Figs. 116 B, 117 A, 118 A). They are often found localized in the haemopoietic centres, particularly in nests of megaloblasts (Fig. 179 A), normoblasts (Figs. 179 B, 180) and plasma cells (Figs. 143, 147), and are then known as **"regional monocytes of the haemopoietic centres".** These monocytes are found in all stages of development—monoblasts and promonocytes, mature cells with loose, lobed nuclei (Fig. 179 B), involution forms with old, degenerate nuclei, and necrobiotic or necrotic disintegrating forms (Figs. 180 C, 181 D). They often contain the remains of ingested or stored material giving a positive reaction for iron or peroxidase (Fig. 179 B). All these properties lend support to the view that these cells of the haemopoietic centres are not multipotent stem cells.

3. **The so-called storage cells** of the storage diseases (thesaurismoses). These must also be classed as monocytes, for in the internal organs where they collect, all forms are to be found, from monocytes with indented nuclei and little power of storage to giant forms with atrophic nuclei. In Gaucher's disease, they contain cerasine, a cerebroside which generally fills the cytoplasm with a felt-like mass of fine needles, but may sometimes be stored in an amorphous, drop-like form (Figs. 119, 120). In Niemann-Pick's disease, the monocytes contain principally phosphatides, while in Hand-Schüller-Christian's disease lipoids, especially cholesterol, are present. In both the latter storage diseases the material is always stored in the form of drops.

4. **Iron-storing monocytes or sideromonocytes** are frequently found in the internal organs. Using specific tests for iron, such as the Turnbull blue (Fig. 115 C) and Prussian blue methods, many phagocytic monocytes may be shown to possess this property of iron storage.

Occasionally, senile storage cells are found containing numerous small spheres of uniform size (Fig. 121 A); the nature of these is unknown. A unique finding is the cell in Fig. 121 B containing long crystalline bodies, some of which have adopted a hair-pin shape owing to the confined space.

THE MONOCYTES IN HEREDITARY ANOMALIES OF THE BLOOD CORPUSCLES

In one of the families in which a **hereditary increase in segmentation of the eosinophil nuclei** was observed (see p. 51), monocytes with segmented nuclei were also present. In the heterozygotic form of **Pelger's anomaly,** the nuclei of the monocytes are less indented than normal. In the homozygotic form in man, 40% of the nuclei are round and 60% indented, while in rabbits all the monocytes have round nuclei. In **Alder's anomaly,** the monocytes, like the eosinophils, basophils, neutrophils and lymphocytes, have a particularly abundant azurophilic granulation. The intensity of the coloration varies, however, suggesting that it may be due to material ingested during phagocytosis (Figs. 181 A, 181 D, 182 H). Some of the monocytes are also affected in **May-Hegglin's anomaly,** the pale blue cytoplasm containing intense blue portions reminiscent of Doehle's inclusion bodies (Fig. 183 C below).

ATYPICAL FORMS OF MONOCYTES

Forms with atypical nuclei. In reactive monocytoses and in leukaemias, the monocytes sometimes have very deeply indented or segmented nuclei. On the other hand, in monoblastic promonocytic leukaemia, the tendency to nuclear indentation normally exhibited in the early stages of development may be absent, and the majority of monocytes may have round nuclei (Fig. 123).

Epithelioid cells. The epithelioid cells found in chronic inflammatory diseases, especially in tuberculosis, appear to be monocytes which have undergone marked degenerative changes under the influence of toxic substances (e.g. the toxins of the tubercle bacillus).

Langhans' giant cells. These multinucleate cells which appear in tuberculosis (Fig. 130) are similar to the giant cells of Hodgkin's disease (Fig. 131) and those caused by foreign bodies. They are thought to be degenerated monocytes but this is not yet certain. Nor has it been established whether they are formed by fusion of several cells following the breaking down of the cell walls, or whether, like the osteoclasts, they are the products of successive mitoses in which no division of the cytoplasm has occurred (polyploid multinucleate cells). The latter explanation seems more probable.

Polyploidy. Like all other blood cells, the monocytes may occasionally exhibit polyploidy. In inflammatory diseases, such as pneumonia, tetraploid binucleate monocytes (twinning deformities) may be found in the peripheral blood (Figs. 126 A, 126 B), while the bone marrow may contain monocytes exhibiting a higher degree of polyploidy (Fig. 126 C), as well as examples of the atypical mitoses which lead to these forms (Fig. 126 B).

DIFFERENTIATION OF THE MONOCYTES

The features which distinguish the monoblasts from other blast cells may be found in Tables 7 to 9, pp. 42 and 43. Cases in which monocytes may be confused with neutrophils have already been discussed on p. 57.

Peroxidase reaction. Using the original method of Graham-Knoll, described on pp. 28 and 29, the monoblasts always give a negative peroxidase reaction. A positive peroxidase reaction is normally given by a few of the promonocytes and by the majority of mature monocytes (Figs 49 D, 50). Using modification I for monocytes (p. 28 and Figs 52 A, 54), practically all the monocytes are negative; a few monocytes are occasionally encountered which give a very slight positive reaction but these create no particular difficulties since the positive reaction of the neutrophils is always much stronger. The positively reacting portions of the monocytes are apparently derived from ingested peroxidase-positive cells, mainly from neutrophils and eosinophils. Evidence for this view is provided by elements such as those shown in Figs 112 E and 113, in which the positive reaction is confined to the still clearly visible remains of the ingested peroxidase-positive cells. As the digestion of the cells proceeds, the peroxidase-positive substance becomes distributed throughout the entire cytoplasm (Figs. 49 D, 50). The rapid inactivation of the peroxidase of the monocytes also points to already degenerated, partly decomposed material from other cells. The atypical neutrophilic promyelocytes or myelocytes found in certain leukaemias often closely resemble monocytes, and modification I of the peroxidase reaction may then be the only certain method of distinguishing the disease from true monocytic leukaemia. The disappearance of the peroxidase from certain of the neutrophils sometimes leads to confusion with monocytes. A method of overcoming this difficulty is described on p. 28 (see also Fig. 94).

Gömöri's method of silver impregnation (see pp. 28, 29). All forms and stages of development of the monocytes are negative. This also holds for all other true blood cells and for the non-haemopoietic elements of the haemopoietic organs, with the exception of the fat and stroma cells (see p. 68 and Fig. 199). In histological sections, the monocytic elements, especially the so-called reticulo-endothelial cells, appear to be surrounded by supporting fibres of reticulin. In touch preparations and films it may be seen, however, that these fibres originate from the stroma cells and not from the monocytes.

EXCESSIVE PRODUCTION OF MONOCYTES

In many diseases, especially those of a chronic, inflammatory nature, there may be a pronounced increase in the number of monocytes in the blood and in the haemopoietic centres (bone marrow, spleen, lymph glands and vascular sheaths). Even though no leucocytosis is present, the monocytes may constitute 50 per cent of the total number of leucocytes in the blood. Infective diseases in certain stages of which a marked monocytosis occurs, include malaria, syphilis and tuberculosis (particularly tuberculosis of the spleen, the lymph glands and the bone marrow). In the diseased organs, the degree of monocytosis may be still greater than in the blood.

Agranulocytosis may be accompanied by marked monocytosis in the blood and the bone marrow. In these cases, modification I of the peroxidase reaction enables confusion with neutrophilic promyelocytes to be avoided (p. 28 and Figs 53, 54). Monocytosis is frequently accompanied by an increase in the other cells formed in the so-called lymphatic tissue—the lymphocytes and plasma cells. Simultaneous lymphocytosis, monocytosis and plasmocytosis in the blood is characteristic of lymphatic glandular fever (Pfeiffer's disease, Fig. 187), while the bone marrow is similarly affected in panmyelopathy (Fig. 188).

In **monocytic leukaemia**, hyperplasia is generally confined to the monocytes. Depending upon the stage of development of the cells released into the blood stream, the leukaemia may be characterized as monoblastic, promonocytic or monocytic, or as a mixed form. The absolute leucocyte count may be over 100,000 per cu mm. Only by means of the peroxidase reaction is it possible to decide for certain whether the cells concerned are monocytes, or whether they are atypical neutrophilic promyelocytes and myelocytes resembling monocytes in appearance. The possibility of a neutrophilic leukaemia with partial disappearance of the peroxidase must be excluded. This can only be done if the bone marrow contains peroxidase-positive neutrophils in all stages of development. As a rule, the monocytes in monocytic leukaemia give a negative peroxidase reaction, even with the original Graham-Knoll method. Some cases of so-called sympathogonioma (*neuroblastoma sympathicum*) are apparently a special form of monocytic leukaemia. In addition to the large, localized tumours found in the upper abdominal region, this disease is characterized by the presence in the blood of cells which frequently contain vacuoles and which may be shown to be atypical monocytes in various stages of development (Figs 213, 214). The disease would thus seem to be a monocytoma accompanied by monocytic leukaemia, a syndrome similar to that of plasmocytoma accompanied by plasma-cell leukaemia. The presence of mature ganglion cells within the tumours may be attributed to the fact that both the sympathetic elements and the centres of monocyte formation are situated in the vascular sheaths.

Occasionally, cases of mixed leukaemias of the "lymphatic monocytic tissue" are seen, analogous to the mixed myelogenous leukaemias which affect simultaneously the basophils, eosinophils and neutrophils. A case of this type, a mixed monocytic and lymphocytic leukaemia, is shown in Fig. 190.

Monocytopenia has not so far been described.

DESTRUCTION

Disintegrating forms of monocytes in the blood and in the haemopoietic organs are particularly frequent in monocytic leukaemias (Figs. 227 E, 228 G, 228 H). The destruction follows the same course as that of other leucocytes. The special, senile forms of athrocytic monocytes found in the tissues have been mentioned on p. 59.

ARTEFACTS

In thin portions of blood and bone marrow films, the monocytes flatten out to a greater extent than other cells, and they therefore appear to be the largest cells of normal blood. In actual fact, however, they are not larger than the neutrophils, and if thicker portions of the film are examined, the two species of cell are seen to have the same diameter (Fig. 109 C). In histological sections, therefore, it almost invariably happens that the monocytes are taken for other cells, unless they contain ingested or stored material. The nuclei of crushed monocytes usually have a broad, boomerang shape (Fig. 233 C).

6. THE SYSTEM OF THE LYMPHOCYTES

Figs 30 centre, 44 C, 44 D, 47 G, 49 B, 51 C above, 52 B centre, 110 F above, 131 to 142, 161 C above, 182 E, 182 F, 187 B, 187 C, 188, 190, 233 A, 234, 238 N

STAGES OF DEVELOPMENT OF THE LYMPHOCYTES

a) **Lymphoblast,** Figs. 47 G, 131 A bottom right, 131 B below, 133 B, 134 A, 135, 136, Tables 7, 8 and 9, pp. 42 and 43

Diameter 12—14 μ This is the stem cell of the system and the smallest stem cell of the leucocytes The nucleus is round and, in contrast to other blast cells, the structure of the chromatin network is only faintly visible The chromatin is arranged in a vague concentric pattern and, though continuous, contains some lumpy concentrations A characteristic feature is that, as a rule, the nucleus contains only a single nucleolus Occasionally, however, two may be present. The nucleolus is of medium size, blue, and somewhat cloudy in appearance In thinly spread lymphoblasts, there is usually little, if any, covering of chromatin and the nucleolus is therefore clearly visible It is surrounded by a moderately wide border of chromatin which merges imperceptibly into the remaining mass of compact chromatin. The cytoplasm has a dark, somewhat uneven, cloudy blue colour, and varies in width. Like all other blast cells, the lymphoblasts are without granulation.

b) **Prolymphocyte,** Figs 131 A (the larger cells in the centre and above), 134 A (the two cells in the centre), 134 B

This stage closely resembles the lymphoblast in appearance, but is sometimes larger and the nucleolus is indistinct. The band of cytoplasm surrounding the nucleus may be very broad There is a continuous transition from the lymphoblast to the lymphocyte, and the classification "prolymphocyte" is without great importance

c) **Lymphocyte,** Figs 30 centre, 44 C above, 44 D above, 49 B, 51 C above, 52 B, 110 F above, 131 to 134, 136 E above, 136 G, 136 H below, 137 to 140, 161 C above, 182 E above, 182 F, 187 B above, 187 C, 188, 190, 233 A, 234, 238 N, Table 6, p 41

Diameter: 9—12 μ. This is the end stage of the system and the smallest leucocyte found in the blood. The nucleus is usually round, but may sometimes be indented, even in healthy persons The chromatin consists of a more or less dense, lumpy mass, and the nucleolus can only be recognized in crushed cells (Fig 234 D) The cytoplasm has a clear, light blue colour and occasionally contains fine, sharply-defined azurophilic granules, surrounded by narrow, clearly visible haloes (Figs 44 D above, 137 A, 137 B) These haloes are a very characteristic feature, since they are not present in other leucocytes with azurophilic granulation.

Mitosis and chromosomes.

The lymphocytes are diploid cells When in mitosis, the lymphoblasts are very liable to be confused with basophilic normoblasts, they can only be recognized with certainty in lymph gland punctate, where mitoses of basophilic normoblasts do not occur The chromosomes are short, thick and only slightly curved (Figs 135, 136) During mitosis the cytoplasm of the lymphocytes separates into two well-marked phases, the basophilic substance becoming granular (Fig 136) A similar phenomenon occurs during the mitosis of megaloblasts and normoblasts (Figs 32, 37, 38), but in the case of the plasma cells, this separation of the basophilic substance is less pronounced (Figs 155, 157) A striking feature of the lymphoblasts is that very few mitoses are found at the sites of lymphocyte formation. This indicates that either mitosis must proceed very rapidly or the life of the individual cells must be very long In reactive lymphocytoses (see Fig 135 A), and in lymphatic leukaemias, mitoses of lymphoblasts may occasionally be found in the blood.

THE LYMPHOCYTES IN HEREDITARY ANOMALIES OF THE BLOOD CORPUSCLES

In **Pelger's anomaly** the lymphocytes also show abnormality, the nuclear chromatin exhibiting a greater degree of aggregation than normally, especially in the homozygotic form. **Alder's anomaly** affects the lymphocytes similarly to the other leucocytes, the azurophilic granulation in some of them being much more abundant than usual (Fig 182 F). **Hurler's disease** (gargoylism) may be accompanied by Alder's anomaly but sometimes only the lymphocytes show coarse granulation In **May-Hegglin's anomaly** the characteristic basophilic streaks in the cytoplasm cannot be detected in the lymphocytes as their cytoplasm is already basophilic

ATYPICAL FORMS OF LYMPHOCYTES

Rieder forms. These lymphocytes with indented nuclei may be encountered even in normal people There may be a marked reactive increase in their numbers in patients with whooping cough (Fig. 139), and a "primary" increase occurs in some leukaemias (Fig 140) In the case of individual cells, it is often difficult to be certain whether they are still diploid with indented nuclei, or whether they are polyploid. When splenopathic depression of the bone marrow progresses to panmyelophthisis, the majority of the lymphocytes may have indented nuclei, both before and after splenectomy

Lymphatic plasma cells. This name is given to very small lymphocytes, the cytoplasm of which is intensely basophilic, like that of true plasma cells. The nuclei of lymphatic plasma cells are markedly pyknotic, a sign of imminent destruction (Figs 44 D below, 132 E) These cells are not true plasma cells, but degenerated lymphocytes They may be seen occasionally in healthy persons and are also a regular finding in Pelger's anomaly, but they have no special diagnostic significance

Giant granulation. In occasional cases of lymphatic leukaemia, lymphocytes may be found containing very large, azurophilic granules (Fig 134 B) In general, however, the lymphocytes in lymphatic leukaemias are characterized by the absence of granulation

Lymphocytes with denuded nuclei. Lymphocyte nuclei practically denuded of cytoplasm are a not unusual finding in lymphatic leukaemia (Figs 133 B, 134 A, 141, 142, 190) Such cells may also be encountered in reactive conditions.

"Atypical cells". A characteristic finding in lymphatic glandular fever is the presence in the blood of giant lymphocytes which have been termed "atypical lymphocytes" or "atypical cells". Their lymphocytic nature is confirmed by the fact that they occasionally contain fine, azurophilic granules surrounded by small haloes (Figs 137 B, 137 C)

Polyploidy. Binuclear lymphocytes (Figs. 138 A to 138 C) and polynuclear lymphocytes (Figs. 138 D) are sometimes found in the blood of healthy persons. In acute leukaemias, lymphocytes with single, giant nuclei may be found (Figs 142 C, 142 D) A pronounced increase in the number of lymphocytes, with many atypical forms, may occur as a reactive manifestation (e g in whooping cough, Fig 139), or may be of "primary" origin (in leukaemias, Fig 140) The only forms which appear to be of diagnostic value in leukaemias are the giant, highly polyploid, mononuclear cells (Fig 142 C, 142 D) and the small lymphoblasts with irregular, "knobbly", almost denuded nuclei (Figs 142 A, 142 B). The atypical mitoses which lead to these forms may also be encountered (Fig 141 B).

Vacuole formation. Vacuoles are seen occasionally in lymphocytes, and are particularly numerous in many of the acute lymphatic leukaemias (Fig. 141).

EXCESSIVE PRODUCTION AND DIMINISHED PRODUCTION OF LYMPHOCYTES

Children often show a marked reactive increase in the number of lymphocytes, and in **whooping cough,** for example, the absolute leucocyte count may exceed 100,000 per cu mm, the greater percentage of these cells being lymphocytes (Fig 139) **In infectious lymphocytosis,** a benign disease of limited duration, the leucocyte count reaches 30,000 to 40,000, the predominant cells being mature lymphocytes The blood picture, therefore, is very monotonous, resembling that seen in chronic lymphatic leukaemia Infectious lymphocytosis differs from the latter disease, however, in its transitory nature and epidemic occurrence and in the absence of general symptoms of leukaemia **Lymphatic leukaemia** generally runs an acute course in children, but tends to be chronic in adults In some cases, the absolute leucocyte count exhibits marked fluctuations, while in others it remains at a constant level, which may be raised, normal or lowered In children who have normal absolute leucocyte counts and no obviously atypical lymphocytes in the blood, it may be difficult to establish a diagnosis without making a marrow puncture The bone marrow will be found to contain greatly increased numbers of lymphocytes, thus confirming the diagnosis It is not infrequent for the leucocytes in lymphatic leukaemia to exceed 100,000 per cu mm. **Lymphatic glandular fever** (Pfeiffer's disease) is characterized by a variegated blood picture, numerous atypical lymphocytes, plasma cells and monocytes being present in addition to normal cells (Figs 137, 187)

Lymphopenia occurs fairly frequently in myelogenous leukaemias and in tuberculosis of the lymphatic system It also appears fairly *frequently during the* treatment of myelogenous leukaemias with urethane and it may then develop into an **alymphocytosis.**

DESTRUCTION

Destruction of the lymphocytes follows the same course as that of other leucocytes In the upper part of Fig 131 B, a lymphoblast in a lymphatic leukaemia is seen in the process of destruction.

ARTEFACTS

Under the influence of external pressure, the lymphocytes are not pressed flat like other blood cells, but are crushed immediately and lose their cytoplasm They are thus converted into the so-called **Gumprecht's shadows** without passing through a Ferrata stage (Figs 47 G, 135 C, 135 D, 234) The cytoplasm either remains in the form of small, blue droplets in the vicinity of the crushed cell, or dissolves in the surrounding blood plasma This peculiar behaviour of the lymphocytes on injury may be useful in establishing a differential diagnosis between acute lymphatic and acute myelogenous leukaemia Droplets of cytoplasm which have been extruded from crushed lymphocytes (Figs 47 G, 234 D, 234 E) should not be confused with pathological, blue blood platelets (Fig 176 D) These droplets are, of course, particularly abundant in lymph gland punctate

Spindle cells, (Fig. 110 F above) are also mechanically produced artefacts, characteristic of the lymphocytes The major axis of these cells generally lies at right angles to the direction in which the film was spread

DIFFERENTIATION OF LYMPHOCYTES

The features by which lymphoblasts may be distinguished from other blast cells are to be found in Tables 7 to 9, pp 42 and 43

Peroxidase reaction. Like the plasma cells, megakaryocytes and platelets, the lymphocytes are invariably peroxidase negative in all stages of development.

7. THE SYSTEM OF THE PLASMA CELLS

Figs. 44 B below, 44 C below, 47 H above, 48, 49 C below, 143 to 180, 182 G, 186 E, 186 F below, 187 A below, 187 B below, 188 B

STAGES OF DEVELOPMENT OF THE PLASMA CELLS

a) **Plasmoblast,** Figs 47 H above, 143 top left, 150 A; Tables 7, 8 and 9, pp 42 and 43

Diameter: approx 16 μ. The stem cell of the system. It has a round nucleus with a dense structure, the arrangement of the chromatin is vague but tends to be concentric It is noteworthy that the nucleoli, of which 3—6 are present, can only be seen with difficulty at this stage, but become clearly visible in the subsequent stage of development The nucleus is situated in the centre of the cytoplasm which forms a narrow border round it No granulation is present.

b) **Proplasmocyte,** Figs 44 B below, 44 C below, 143 below, 145, 146, 148 A, 148 B, 150 B, 151, 153, 160 A

The proplasmocyte may reach 25 μ in diameter and is therefore considerably larger than the plasmoblast. The nucleus is often still situated centrally, but may be slightly eccentric It has

an indistinct chromatin network containing several blue nucleoli. These are generally uncovered and are surrounded by a narrow border of chromatin. The cytoplasm has a deep blue colour which is usually sufficiently intense to mask the slight red component. Surrounding the nucleus is a pale halo known as the archoplasm. Even under normal conditions, vacuoles are occasionally present at this stage (Figs. 145 A, 145 B).

c) Plasmocyte, Figs. 47 H below, 49 C, 143 above, 144, 147, 149, 150 C, 151, 152 A, 156, 160 B, 186 E, 186 F above, Table 6, p. 41.

Diameter: 14 to 20 μ. The cell is often elongated and the nucleus is eccentric and relatively small. The chromatin structure is coarse and dense and has the so-called "cartwheel" arrangement. Even in crushed cells, the nucleoli can no longer be seen. The masked red component is sometimes weakly visible through the deep blue of the cytoplasm. There is often a wide halo of archoplasm closely surrounding the nucleus (Figs. 144, 147, 151).

Granulation.

No granulation is present in normal plasma cells.

Nucleoli.

In the plasmoblasts, the nucleoli can only be seen with difficulty, but in the proplasmocytes they become clearly visible. The plasmocytes often contain dense, post nucleolar chromatin masses.

Mitosis and chromosomes.

The plasma cells are normally diploid cells. Mitoses are a comparatively rare finding. The chromosomes are thick and slightly curved, in the later stages of development, they have a greater tendency to aggregation than the chromosomes of other blood cells. Separation of the cytoplasmic constituents during mitosis is not so marked as in the lymphocytes (compare Fig. 157 A with Fig. 136).

The mitosis of plasma cells may occasionally be observed in the blood in normal people, Fig. 155.

THE PLASMA CELLS IN HEREDITARY ANOMALIES OF THE BLOOD CORPUSCLES

As the plasma cells constitute only a small proportion of the total number of blood corpuscles, little is yet known regarding their behaviour in these anomalies. In the homozygotic form of **Pelger's anomaly,** the chromatin of the plasma cells is even more abundant and lumpy than normal. The plasma cell is the only species of blood cell which does not exhibit granulation in **Alder's anomaly** (Fig. 182 G).

ATYPICAL FORMS OF PLASMA CELLS

Vacuoles. Even under normal conditions, plasma cells often contain several vacuoles. In small, thin vacuoles, the contents appear colourless, but in larger and thicker vacuoles, the contents may be bluish or reddish, the depth of colour depending on the concentration. In many cases of plasmocytoma, and also in reactive conditions, the vacuoles may become very large (Figs. 48 B, 152 A). Finally, on destruction of the cell, the contents of the vacuoles may be liberated and fuse together to form "Russell bodies". These can be found not only in histological sections, but also in bone marrow films (Fig. 152 B).

Inclusions. Plasma cells contain no granules. It is therefore all the more striking that in very rare cases of plasmocytoma (Fig. 153 A), the plasma cells contain needle-shaped (occasionally rhomboid) azurophilic inclusion bodies. These bodies are very similar in appearance to the Auer rods found in neutrophilic promyelocytes.

During the treatment of plasmocytoma with amidines (stilbamidine, pentamidine), coarse, lumpy granules may appear in the cytoplasm of the plasma cells (Fig. 153 B). These consist of the amidine combined with ribonucleic acid and are known as Snapper-Schneid inclusion bodies.

"Flaming" plasma cells. In certain diseases, the red component of the cytoplasm, which is normally masked by the deep blue colour, may predominate and appear in the form of large, broad, red streaks (Figs. 149 A, 149 B). This is especially frequent in the presence of a reactive increase in the number of plasma cells, e.g. in agranulocytosis. It is necessary, of course, to ensure that the staining technique has not been at fault.

Plasma cells with segmented nuclei. In occasional cases of plasmocytoma, a more or less large number of plasma cells may develop segmented nuclei (Fig. 148 C, 154). This is apparently [illegible]

ments which still have the shape of chromosomes. This phenomenon strongly recalls the changes which can be produced by mitotic poisons such as colchicine.

[illegible]

support for the theory that the plasma cells do not belong to the true blood cells, but to the fixed cells of the tissues where polyploidy is common. The simultaneous occurrence of atypical mitoses (Figs. 157, 158 B) indicates the mitotic or endomitotic origin of these polyploid cells.

EXCESSIVE PRODUCTION OF PLASMA CELLS

The plasma cells normally occur only in small numbers in the blood, constituting scarcely one per cent of the total leucocyte count. On the other hand, in the bone marrow they are remarkably frequent, and are often arranged in nests containing one or two "regional monocytes" (see p. 59 and Figs. 143 and 147). It therefore appears that the isolated plasma cells which normally find their way into the circulation are only those which become detached accidentally while proliferation is in progress. Generally, they are proplasmocytes bearing evidence of degen- [illegible]

[illegible] number. The nests of plasma cells in the bone marrow are usually more numerous in diseases with reactive plasmocytosis, such as rubella, and are particularly abundant in lymphogranuloma inguinale. A reactive increase in the plasma cell nests may

easily be overlooked when making bone marrow counts under oil immersion, and is best detected if the preparation is examined under the low power. In certain diseases, e.g in splenopathic depression of neutrophilopoiesis, a marked reactive increase in the plasma cells of the bone marrow occurs. This may be distinguished from the primary hyperplasia seen in plasmocytoma by the fact that the plasma cells remain localized in nests, whereas in advanced plasmocytoma diffuse infiltration takes place

Plasmocytoma and plasma cell leukaemia. Owing to the fact that plasma cells properly belong to the fixed cells of the tissues, so-called "primary" diseases of the plasma cells usually manifest themselves as true malignant tumours, without the development of a leukaemic condition in the blood. The modern view is that "multiple myeloma" (Kahler's disease) is invariably due to excessive proliferation of the plasma cells; the tumour form of the disease is therefore known as "plasmocytoma", and the rare form which is accompanied by the discharge of plasma cells into the blood stream, as "plasmocytoma with plasma cell leukaemia". Like lymphatic leukaemia, and the leukaemias which affect exclusively the neutrophils, eosinophils, basophils or megakaryocytes, plasma cell leukaemia is a primary affection confined to a single species of cell. Occasionally, young cells belonging to other [illegible]

[illegible]

[illegible]

ment of the plasma cells principally affected, the plasmocytoma or leukaemia is termed plasmoblastic, proplasmocytic, or plasmocytic (see Figs 150, 151)

For the diagnosis of plasmocytoma, it is important to know that the vast majority of patients show changes in the blood protein values which are pathognomic (Wuhrmann and Wunderly 1952, see "Literature" p. 77) These changes are similar to those seen in macroglobulinaemia (Waldenström) but a differential diagnosis is made possible by the fact that there is no marked increase in the plasma cells in the latter case, although a lymphocytosis is present.

Plasmocytopenia. A clinical symptom corresponding to plasmocytopenia has not so far been described

DESTRUCTION

The destruction of the plasma cells does not differ in any way from that of other blood corpuscles

ARTEFACTS

The plasma cells are extremely fragile and in thin films or in touch preparations their cytoplasm readily escapes, often forming long filaments

DIFFERENTIATION OF PLASMA CELLS

Like all other species of blood corpuscle, the plasma cells form a system of their own and have their own stem cells. The distinctive features of the plasmoblast may be found in Tables 7, 8 and 9 on pages 42 and 43. It should be especially noted that the plasmoblast possesses three to six nucleoli, whereas the lymphoblast has only one.

Plasma cells are liable to be confused with osteoblasts, but the latter are larger and the cytoplasm has an irregular outline as the cells are derived from tissue structures Moreover, the archoplasm of the osteoblasts is at some distance from the nucleus instead of in direct contact with it as in the plasma cells (compare Fig. 144 with Fig 201).

Peroxidase reaction. Like the lymphocytes, megakaryocytes and platelets, the plasma cells are peroxidase negative in all stages of development.

Unna-Pappenheim staining. This staining may be used not only for histological sections but also for blood and bone marrow films. It was claimed to be specific for the recognition of plasma cells. As Fig 48 A shows, the plasma cells do, indeed, give an intense coloration, and it is of interest that the Russell bodies also stain in a like manner (Fig 48 B). The method is not specific, however, as many blast cells, including the more mature erythroblasts, likewise stain deeply, and so do the granules of the eosinophils and tissue basophils (Fig 48 A) This method has, therefore, not been included in the section on the staining of films

8. THE SYSTEM OF THE MEGAKARYOCYTES AND PLATELETS

Figs 21 C, 47 I, 161 to 178, 184, 240 F below

STAGES OF DEVELOPMENT OF THE MEGAKARYOCYTES AND PLATELETS

a) Megakaryoblast, Figs 47 I, 161 to 164 H, Tables 7, 8 and 9, pp 42 and 43.

The term megakaryoblast is applied generally to all young cells of the system having ungranulated cytoplasm which stains a pure blue with no red component. Depending upon their degree of maturation, up to three different stages of development or generations of megakaryoblasts may be distinguished These differ from one another in the size of the cell and nucleus and, sometimes, in the number of nuclei as well.

The *diploid megakaryoblast* is the stem cell of the system (Figs 47 I, 161 A, 161 B) It has a diameter of approximately 20 μ. The nucleus is round, and the chromatin is somewhat scanty and without characteristic arrangement. The chromatin network is ill-defined and made up of fine, elongated fibres It contains several nucleoli which are small and very indistinct. Some are covered with chromatin through which they glisten pale blue, they have no well-marked chromatin border The cytoplasm is moderately basophilic. It forms a more or less broad band round the nucleus and often has tongue-shaped or fringe-like processes.

The *tetraploid megakaryoblast* is considerably larger and is formed from the diploid stage without division of the cytoplasm When karyokinesis is accompanied by division of the nucleus, a

cell with two diploid nuclei is produced (Fig. 162 D), if the nucleus remains undivided (endomitosis), a cell with a single large, tetraploid nucleus is formed (Fig. 162 E)

The *octoploid megakaryoblast* is the next stage of development. This again arises from the preceding stage by mitosis without division of the cytoplasm. Depending upon the type of karyokinesis which takes place, it may have four diploid nuclei (Fig 163 F), two tetraploid nuclei, two diploid nuclei and one tetraploid nucleus, one diploid and one hexaploid nucleus, or only a single giant octoploid nucleus (Fig 163 G) The nucleus has a somewhat more compact structure than in the preceding stages of development.

b) Promegakaryocyte, Figs 164 I, 165, 167, 168 A 171 A.

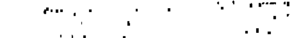
Maturation of the megakaryoblast leads to the formation of the promegakaryocyte. It contains a characteristic polychromatic or oxyphilic material which may be either localized or diffused throughout the cytoplasm. As a rule, the promegakaryocyte has only one nucleus since karyokinesis takes place principally by endomitosis, both in the case of the promegakaryocyte itself, and in the older blast cells, even in the polynuclear forms of the earlier stages of development, the chromosomes tend to regroup themselves to form a single nucleus (Figs 164, 165 K) The nucleus is usually more or less indented. It is improbable that it ever becomes more than 16- or 32-ploid.

c) Megakaryocyte, Figs 166, 178 B

Diameter: approx. 100 μ This stage of development of the system is the last which still constitutes a complete cell. It is formed by maturation of the promegakaryocyte after it has lost the power of karyokinesis. The nucleus usually has numerous indentations and is not segmented, in appearance it often resembles the antlers of a stag. In thin, weakly stained preparations, very numerous, small blue nucleoli are visible in the relatively dense chromatin. The cytoplasm is pink and contains fine, azurophilic granules In cells which have not completely matured, the granulation is diffused throughout the entire cytoplasm. In fully mature cells, the granules lie together in small groups (granulomere) separated from one another by areas of ungranulated cytoplasm (hyalomere).

d) Blood platelet, Figs 170, 172 E, 172 F, 173 to 176 D, 177, 240 F below.

Diameter: 1—2 μ The blood platelet is the definitive form of this cell system. It is merely a fragment of cytoplasm from the megakaryocyte, consisting of the azurophilic granulomere surrounded by a narrow border of ungranulated hyalomere



development, the platelet, consists only of a fragment of cyto-plasm. All other cells normally remain diploid during their development, polyploidy being an exception, and their final stages are still complete cells which may be either nucleated (leucocytes) or non-nucleated (erythrocytes)

Granulation.

Progranulation is not observed in this system The azurophilic granules which appear at the promegakaryocyte stage constitute the final granulation During the process of maturation they increase in density so that, by the time the platelet stage is reached, they are very coarse and dark in colour

Mitosis and chromosomes.

The chromosomes of the megakaryoblasts and promegakaryocytes are comparatively long and thick, and the angles are obtuse. When the cells are well spread out, the individual chromosomes may be clearly distinguished. In the more mature stages of development, where the degree of polyploidy is great, large numbers of chromosomes are present (Figs. 162 C, 164, 165 K).

THE MEGAKARYOCYTES AND PLATELETS IN HEREDITARY ANOMALIES OF THE BLOOD CORPUSCLES

In the heterozygotic form of **Pelger's anomaly,** the nuclei of the megakaryocytes are less indented than normal; in the homozygous form, the nuclei are round. In **May-Hegglin's anomaly,** the cytoplasm of the mature megakaryocytes is divided up into comparatively large areas of granulomere (Fig. 184 D) and, consequently, giant platelets are found in the circulating blood (Fig. 184 E). The megakaryocyte-platelet system has not so far been found to participate in any of the other anomalies.

ATYPICAL FORMS OF MEGAKARYOCYTES AND PLATELETS

Vacuoles. Vacuoles may be found in the cytoplasm of the megakaryoblasts and the promegakaryocytes, and the mechanically produced cytoplasmic processes of these cells are sometimes filled with tiny vacuoles (Fig. 165 L). These cells may also contain large rosette-shaped vacuoles of unknown content (Figs. 165 K, 167), similar to those seen in other species of leucocytes (Fig 106 A)

Hyaline promegakaryocytes (Fig. 167). Hyaline promegakaryocytes are pathological forms found in the bone marrow in certain irritant conditions of the system They are relatively large, fairly mature forms, characterized by the fact that the cytoplasm has a clear blue colour and is still largely, if not entirely, ungranulated. In other words, certain properties of the early stages of development persist in the more mature forms.

Hypersegmentation. In pernicious anaemia, the hypersegmentation of the nucleus observed in the neutrophils, eosinophils and basophils of the blood may be accompanied by a similar phenomenon in the promegakaryocytes and megakaryocytes of the bone marrow (Fig. 168).

Polynuclear megakaryocytes. Under certain conditions, some of the promegakaryocytes undergo mitosis without the chromosomes uniting to form a single, large nucleus, and megakaryocytes with numerous small nuclei are produced (Fig 171). This is especially likely to occur where relative anoxaemia is present, as in the embryo and foetus and in patients with decompensated heart failure The individual nuclei vary in size and are not necessarily all diploid, as the distribution of the chromosomes is uneven, some of the nuclei are connected by bridges It is not always easy to distinguish such polynuclear megakaryocytes from highly segmented specimens (cf. Fig 168), since the individual segments of the latter may break loose and be mistaken for single nuclei The nuclei of polynuclear megakaryocytes are sufficiently small to pass through the lung capillaries and therefore find their way into the peripheral blood, as in myelogenous leukaemia (Fig 172)

Deformities. The final degree of polyploidy of the megakaryocytes appears to be fixed, development ceasing after a certain number of mitoses have taken place and the size of the cell then remaining stationary. Very rarely, however, particularly large megakaryocytes are found containing two separate nuclei, each of normal size (Fig 178 A). These monster forms ("twinning deformities") probably develop from very young cells in which a diploid mitosis takes place without division of the cytoplasm

Pathological platelets. Fragments of cytoplasm derived from immature megakaryocytes are known as "blue platelets". They are particularly frequent in the blood of patients suffering from an essential or reactive thrombopathy or from myelogenous leukaemia (Fig 176 D) "Blue platelets" should not be confused with Hittmair's particles, which are fragments of cytoplasm mechanically detached from leucocytes, usually from lymphocytes (Fig. 234) Other platelets which must be considered pathological are the ungranulated or only slightly granulated platelets frequently found in myelogenous leukaemia (Fig 175 B) Giant platelets (Figs 176 A to 176 D) may be merely portions of normal megakaryocytic cytoplasm which have not yet broken down to their final size, or they may be derived from megakaryocytes in which the areas of granulomere are very large (Fig 184 E). In the latter case, further reduction in size does not occur. Increased number of large platelets are found in the blood in many reactive affections of the bone marrow, particularly those of an inflammatory nature, and in myelogenous leukaemias (Figs 176 A, 176 D).

Fragments of megakaryocyte nuclei in the blood. Normally, fragments of megakaryocyte nuclei are very rare in human blood (Fig. 176 E) though they are a frequent finding in the blood of many animals. Increased numbers of such nuclear fragments are invariably present in chronic myelogenous leukaemia (Figs 177 B, 177 D), and the same phenomenon is sometimes observed in inflammatory diseases (Figs. 177 A, 177 C) It is sometimes difficult to distinguish between small nuclei and detached nuclear segments (cf. Fig 172 with Fig. 177) Provided that the coagulation of the blood is normal, the nuclear fragments and the small nuclei both agglutinate with the platelets (Figs 172 E, 172 F, 177 A to 177 C).

EXCESSIVE PRODUCTION AND DIMINISHED PRODUCTION OF MEGAKARYOCYTES AND PLATELETS

In purpura haemorrhagica (essential thrombocytopenia or Werlhof's disease), a form of splenopathic depression of the bone marrow characterized by a greatly reduced platelet count, the bone marrow may contain very large numbers of megakaryocytes They collect in large nests containing many young forms, cells in mitosis, senile cells and necrobiotic forms Apparently, the breakdown of the cells to form platelets is inhibited Removal of the spleen overcomes this defect and is followed by the release of large numbers of platelets into the blood stream. A simultaneous decrease in the platelet count and in the number of megakaryocytes in the bone marrow is seldom a primary disorder but usually indicates a reactive process, most often of toxic origin

Megakaryocytic leukaemia is sub-divided into two forms One form is characterized by the appearance in the blood of large numbers of megakaryocyte fragments and immature stages of other cells This is probably only a special form, or an early stage, of chronic myelogenous leukaemia, which sometimes affects the megakaryocytes as well. In the second form (Fig 170), the increase is confined to the megakaryocytes of the bone marrow and is even greater than that in purpura haemorrhagica. At the same time, the number of platelets in the circulating blood may reach 2 to 3 million per cu mm The majority of the megakaryocytes show qualitative changes — the nuclei are inclined to be less highly segmented and the cytoplasm remains blue In bone marrow films, masses of agglutinated platelets may be seen, a phenomenon not encountered in thrombocytopenia

DIFFERENTIATION OF MEGAKARYOCYTES AND PLATELETS

The features by which megakaryoblasts can be distinguished from other blast cells will be found in Tables 7, 8 and 9, pp 42 and 43.

In the examination of bone marrow films, an important practical point is the differentiation between megakaryocytes and osteoclasts. The megakaryocytes have a single nucleus consisting of a number of segments of various sizes joined by bridges, the nucleoli are small and often completely invisible. The cytoplasm is filled with fine granules of uniform size which may be evenly distributed or arranged in a patchwork. Often the megakaryocytes are agglutinated with blood platelets Even in pathological, polynuclear megakaryocytes, the nucleoli are not visible, while the nuclei are often unequal in size and sometimes connected by bridges. The osteoclasts, on the other hand, have numerous small, round nuclei, all equal in size, without connecting bridges Each nucleus contains a single, large nucleolus The granules are variable in size, often of gravel-like appearance, and irregularly distributed throughout the cytoplasm The osteoclasts never agglutinate with blood platelets, although masses of agglutinated platelets are sometimes brought into contact with large osteoclasts during preparation of the film They then give the appearance of agglutination, but, in fact, this is a purely mechanical effect Care should be taken to avoid confusion between blood platelets and Howell-Jolly bodies or malarial parasites Figs 173 C, 240 F).

Peroxidase reaction. The megakaryocytes and platelets are invariably peroxidase negative

Feulgen reaction. The nuclei of megakaryoblasts, promegakaryocytes, and megakaryocytes are Feulgen positive, showing that they contain thymonucleic acid (Fig 21 C) The cytoplasm of the megakaryocytes and platelets gives a negative Feulgen reaction. Like the cytoplasm of all other mature cells, it contains only purine bodies resulting from the enzymatic decomposition of nuclear substances These no longer give a positive reaction and are expelled into the cytoplasm when the maturing nucleus decreases in size

DESTRUCTION

The nuclei and cytoplasm of the megakaryocytes and platelets, unlike those of other species of leucocytes, are not destroyed simultaneously, nor, as in the case of the erythrocytes, does the destruction of the nucleus precede that of the cytoplasm

Instead, the nucleus separates from the megakaryocytes and its destruction follows either in the haemopoietic centres themselves, or in the pulmonary capillaries. The remaining cytoplasm enters the circulation in the form of blood platelets, which are destroyed in the blood when their function has been fulfilled.

ARTEFACTS

The cytoplasm of the megakaryocytes and platelets has very little mechanical strength. At the same time, both elements possess the capacity of agglutination, and they are therefore more susceptible to artificial deformation than any other species of blood corpuscle. The larger cell forms, the megakaryoblasts and megakaryocytes, are more readily damaged than the small ones.

Damaged megakaryocytes. Even when bone marrow films are prepared using the most careful technique, practically all the megakaryocytes are damaged. In some cases, the cytoplasm is pulled out in the manner shown in Figs 161 to 165, while in others, the nucleus is separated from the cytoplasm or the cell is torn into several fragments. A detached fragment is shown in Fig 184 D.

Agglutination. When coagulation sets in during the preparation of a blood film, the blood platelets agglutinate to form more or less large clots (Fig 174). Similarly, in bone marrow films, the platelets agglutinate with megakaryoblasts, promegakaryocytes, megakaryocytes and detached fragments of their nuclei and cytoplasm. The so-called "platelet-forming megakaryocytes" are therefore agglutinates of megakaryocytes with platelets derived from blood contaminating the sternal marrow. Although it is true that the platelets are derived from megakaryocytic cytoplasm, as a rule they are formed only in the peripheral blood from the larger detached fragments.

Fibrin fibres. These are seen attached to the platelets in deeply stained blood films prepared when coagulation is already well advanced (Fig. 175 A). Otherwise fibrin fibres are generally chromophobic.

Star forms. As already mentioned, the blood platelets are very easily damaged mechanically, though they are very resistant to spontaneous decomposition. The preparation of blood films, and even the simple covering of a suspension of blood platelets with a cover slip, is sufficient to rupture a large number of platelets and produce star-shaped forms. If, however, the suspension is allowed to dry in the form of a thick drop, the platelets are not damaged.

Pseudo-phagocytosis. Megakaryocytes and other blood cells may become superimposed on one another during the preparation of blood and bone marrow films. Moreover, the promegakaryocytes and megakaryocytes are large cells with deep indentations in their cytoplasm, in which they readily trap other blood corpuscles. This phenomenon, which is easily observed in histological sections, is not a true phagocytosis but an artefact, though the imprisoned cells may even show evidence of decomposition, since they are unable to escape and gradually degenerate. However, their substance does not pass into the cytoplasm of the megakaryocytes, as would be the case in true phagocytosis such as exhibited by the monocytes. This is proved by the fact that the cytoplasm of the megakaryocytes never gives a positive peroxidase reaction, even after disintegration of imprisoned, peroxidase-positive neutrophils.

V. Cellular Elements Found on Marrow Puncture but not Belonging to the Haemopoietic Systems

Figs. 191 to 226

1. CELLS PROPER TO THE BONES AND BONE MARROW

Tissue basophils (basophils with insoluble granulation, tissue mast cells, heparinocytes), Figs. 188 B, 191, 192.

Diameter: in thick films, approx. 15 μ, in thinly spread out films, up to 25 μ. This species of cell does not normally occur in human blood, but an exception has been reported (see p 19). A blast cell without granulation is unknown, but several granulated stages of development can be distinguished. The very young cells have a single large nucleus with a compact, fine, indistinct chromatin network; the cytoplasm contains only a few basophilic granules There is also a second juvenile stage in which the nucleus is still very large but numerous granules fill the cytoplasm (Fig 191 A). In the mature stage, the nucleus is still round but is small and pyknotic (Figs. 191 C to 192 H). The cytoplasm contains numerous small granules of uniform size, which show an intense metachromasia with toluidine blue (Fig 192 H) If only weakly stained, the nucleus appears paler than the granulated cytoplasm (Fig 191 C, 192 F) The mitoses are characterized by the marked aggregation of the chromosomes which cannot be recognized individually (Fig 191 B).

The differences between tissue basophils and blood basophils are described on pages 9 and 50.

Tissue basophils have not so far been found in normal bone marrow, but appear in the marrow in disturbances of haemopoiesis, especially in diseases of the neutrophil system. They are particularly numerous in essential panmyelopathy (Fig 188) and also occur in May-Hegglin's anomaly, lymphatic leukaemia, agranulocytosis and splenopathic depression of the bone marrow.

Vascular cells (endothelial cells and muscle cells of the vessels), Figs. 193 to 196.

The bone marrow is richly supplied with vessels Consequently, sternal marrow always contains fairly large numbers of vascular cells As these cells are in tissue formation, they are generally severely damaged during aspiration of the marrow and preparation of the film In thin portions of the film especially, only more or less denuded nuclei can be seen, and it is then difficult to say whether they derive from endothelial or muscle cells When their tissue relationships have not been disturbed, the vascular cells are elongated (Figs 193, 194). Isolated cells remain spindle-shaped if derived from the larger vessels, but become round when separated from smaller, thin-walled vessels, such as the venous sinuses (Figs. 194 right, 195, 196). When well spread out, the nuclei have a diameter of 10—15 μ, their chromatin network is uncharacteristic and rarely contains nucleoli. As might be expected from their original tissue formation, detached endothelial cells are generally seen lying in more or less large groups. The vascular cells of bone marrow films (Fig 195) are indistinguishable from the vascular cells occasionally found in films prepared from digital blood (Fig 196), and from the vascular cells of other organs found in touch preparations.

Stroma cells (fat cells), Figs. 197 to 200

The stroma or fat cells are as widely distributed throughout the bone marrow as they are elsewhere in the organism, and act as supporting and interstitial tissue. They are the largest diploid cells and are composed of a thin membrane of cytoplasm which encloses a large fat vacuole and is traversed by true reticulin fibres (Fig 199) The nucleus may be round or slightly oval (Figs 197 to 199) The chromatin network has a coarse mesh and the nucleoli are not usually visible. Immediately surrounding the nucleus is a star-shaped zone of polychromatic cytoplasm, while the peripheral zone is oxyphilic. The division between the two zones is less marked in man than in certain animals (e g in the rabbit) A number of pigmented granules are often seen lying near the nucleus The individual stroma cells are connected to one another by filament-like processes to form a network or reticulum Since they are the only cells of the haemopoietic organs which are joined together in this way and which contain reticulin fibres, they are, strictly speaking, the only cells to which the term "reticulum cells" should be applied. The name stroma cell is to be preferred, however, as it is more descriptive of the properties of the cell and does not create the impression that it is a kind of multipotent stem cell.

In fibrosis of the bone marrow, such as occurs in rickets, the cytoplasm is thickened and the reticulin fibres are more numerous (Fig 200) In cachectic patients, the fat vacuoles are absent, their place being filled by the swollen cytoplasm containing a loose mass of tangled reticulin fibres. This results in the production of a so-called "gelatinous marrow" or "bone marrow oedema"

Owing to their pouch-like shape and the almost liquid nature of their contents, the stroma cells are nearly always damaged on aspiration of the sternal marrow and in the preparation of the film The cytoplasmic envelope is torn apart like a paper bag, allowing the fat to escape. The collapsed cells, being covered in fat, do not adhere well to the glass and are readily washed away or wiped off

Osteoblasts, Figs 201, 202

The osteoblasts are elongated cells, the major axis of which measures up to 35 μ and the minor axis 20 μ. The nucleus may be round or slightly elliptical and the chromatin is relatively abundant with a coarse network. It usually contains two, and occasionally three nucleoli, but sometimes only one is present. In thinly spread cells, the nucleoli appear blue and of medium size If the cells are not very thinly spread, the cytoplasm has a deep blue colour and contains a circular, polychromatic zone of archoplasm at some distance from the nucleus The immature forms are small and have clearly visible nucleoli, while the more mature forms have more cytoplasm but smaller nuclei Mitotic forms are also seen occasionally, the thick chromosomes being

relatively easy to distinguish from one another (Fig 202 B) The contours of the cytoplasm are not so sharply defined as in the blood corpuscles, owing to the fact that the osteoblasts are normally joined together in the form of compact tissue which is torn apart in the preparation of the film

Once the characteristics of the osteoblasts are known, they should not readily be confused with plasma cells They may be recognized by their larger size and by the fact that their archoplasm is situated at some distance from the nucleus instead of in direct contact with it. Moreover, some of the plasma cells contain vacuoles, and these are never present in intact osteoblasts. As the osteoblasts are tissue cells and are generally in tissue formation, it is possible that they may be mistaken for tumour cells.

Osteoclasts (Howell's polykaryocytes), Figs 203 to 206

The osteoclast is the largest cell encountered in films of the haemopoietic organs. It is a polynuclear, highly polyploid cell, each nucleus being diploid. The chromatin is scanty and has a sponge-like structure with a well-defined border. The nucleus usually contains a single, clearly visible nucleolus surrounded by a narrow border of chromatin. If the film is not too thick, the nucleolus appears blue Occasionally, two nucleoli are present The cytoplasm is pale blue and often has a fine, azurophilic granulation, thus having a certain resemblance to the cytoplasm of the megakaryocytes Sometimes, however, it contains groups of azurophilic bodies of various sizes, some being very coarse These appear to be degradation products of bone substance (Fig 205) In bone marrow films only fragments of osteoclasts are found, but these may measure up to 100 μ in diameter Depending upon the size of the fragment, the number of nuclei may vary from only a few up to more than a hundred. The largest fragments are those obtained on post-mortem examination when the cells have become detached from their surroundings and are less liable to be torn (Fig 203).

The number of osteoclasts in the bone marrow is increased in several diseases of the skeletal system, especially in *osteitis fibrosa cystica generalisata* (von Recklinghausen's disease), where the foci contain large numbers of these cells, and in certain bone sarcomas (Figs 207, 208) In rare, reactive deformities of the osteoclasts, giant nuclei may be found which have failed to divide during karyokinesis, having undergone endomitosis (Fig 206) In osteoclast sarcomas (Fig 207) these cells may adopt the most bizarre, polymorphous forms, and the corresponding atypical mitoses may also be found (Fig. 208)

Osteoblasts and osteoclasts are the cells responsible for bone growth and are therefore abundant in bone marrow films and bone sections from embryos, foetuses and children The bones of young embryos at first contain only osteoblasts and osteoclasts Haemopoiesis in the bone marrow does not begin until later, and even then the bone marrow films are found to contain very large numbers of osteoblasts and osteoclasts mixed with young blood cells Both types of cell are rare in adults; they are absent entirely from all extra-medullary haemopoietic organs and from myelogenous metaplasias. The true nature of the osteoblasts and osteoclasts in bone marrow films was for a long time unrecognized The osteoblasts were usually regarded as "reticulum cells" and, owing to their resemblance to plasma cells and their tissue formation, were thought to be "reticular" stem cells of the plasma cells The osteoclasts, on the other hand, were assigned to the megakaryocyte system

2. CELLS FOREIGN TO THE BONES AND BONE MARROW

ELEMENTS INTRODUCED AS THE RESULT OF PATHOLOGICAL CONDITIONS

Tumour cells, Figs 209 to 220, 231

In 25 per cent of patients suffering from malignant tumours, metastatic tumour cells may be found in the bone marrow They are usually easy to recognize, since, being tissue cells, they occur in fairly large groups, most often seen lying near the edges or ends of the film Tumour cells may vary greatly in appearance and rarely enable the nature of the primary tumour to be [illegible]

[illegible] defined outlines and the more or less marked [illegible]

[illegible] tin is sometimes very distinct but may also be [illegible] cytoplasm which forms a band of varying width round the [illegible] and is [illegible]

True sympathogonioma cells (Fig 212), [illegible] from compact tissue, do not have well-defined borders when seen in bone marrow films, nor can they be detected in the circulating blood. They differ in this respect from the metastatic cells of monoblastoma, for, although this is also a localized tumour, the cells resemble all other blood cells in having an intact, sharply outlined cytoplasm (Fig 213), and in finding their way into the peripheral blood (Fig 214)

Metastatic melanosarcoma cells can easily be recognized by their dark pigment (Fig 215), unless they are present in the leuco-form (Fig 216), when they must be identified by other criteria.

Tumour cells in the process of destruction are to be found in metastases containing zones of necrosis (Fig 232)

In patients with metastatic tumours, blood from the ear lobe, where the monocytes are found in large numbers, may also contain a few large peroxidase-negative elements, not normally found in the blood These are probably tumour cells They can only be found if the ear lobe is not handled before puncture Films prepared from the first two drops are stained and examined first under the low power, especially at the edges. In view of the possibility of mistakes and the serious prognosis of malignant tumours, however, a positive finding should not be considered sufficient in itself to establish a diagnosis, but should only be employed as confirmatory evidence

Lymphogranuloma cells, Figs. 217 to 220

In cases of lymphogranuloma, preparations obtained by puncture of the affected lymph glands contain the characteristic giant cells described by Sternberg These are large, highly poly-

ploid elements, the nuclei of which have a coarse chromatin network and sometimes contain characteristic giant nucleoli of a brilliant blue colour. The cytoplasm is often basophilic and usually damaged. It is still not known with certainty from which normal species of lymph-gland cell these pathological giant forms are derived Their origin may in some cases be traced to giant chromosomes formed during karyokinesis by doubling of the parent chromosomes without subsequent division. This is confirmed by the enormous size of the nucleoli and the relatively coarse structure of the chromatin

ELEMENTS INTRODUCED INTO THE MARROW DURING PUNCTURE

In its passage through the skin and subcutaneous tissues, the puncture needle may carry with it cutaneous and other elements, even when protected by a mandrin. The most important of these elements, which may contaminate the marrow specimen, are shown in Plate 39, Figs 221 to 226, and described in the accompanying text as well as on p. 19

VI. Destruction of the Blood Cells

Pp 10, 11, 17 and 18; Figs. 131 above, 180 and Plate 40

The liquefied nucleus of disintegrating cells behaves in a manner reminiscent of mercury and forms either a single round drop or several small droplets If it is very viscous, it may be distorted and have a streaky appearance Segmented nuclei give rise to at least as many droplets as they had segments, but sometimes several droplets may coalesce to a single large drop On [illegible] terial continues to give a dark violet colour with panchromatic stains, it still gives a positive Feulgen reaction Cells undergoing destruction and still containing basophilic chromatin are termed *necrobiotic* (Figs. 131 B above, 180 C, 227 to 231 B, 232); when basophilic chromatin is no longer present, they are said to be *necrotic* (Figs 180 D, 231 C). The destruction of the blood corpuscles proceeds in a fundamentally similar manner to that of other cells.

In leukaemias, it is not uncommon to find necrobiotic and necrotic forms of young cells, clear evidence that the leukaemic cells are unable to live and develop Even in blood diseases which are not of a "primary" nature, however, destruction of immature cells may sometimes occur, as, for example, in the case of the megaloblast in pernicious anaemia (Fig 231 A) Disintegrating forms of leucocytes are found in large numbers in pus. They may also be obtained artificially if a film is prepared from the leucocyte layer of a sample of citrated blood which has stood for 48 hours in the sedimentation tube (Fig 230).

The peculiar disintegrating forms found in acute disseminated lupus erythematosus are shown in Figs 185 and 186 (see also p 18)

VII. Artefacts of Cellular Elements of the Blood and Bone Marrow

P. 11, Plate 41, and other illustrations

Mechanically damaged and deformed cells constitute an almost inexhaustible source of error in interpreting blood and bone marrow films.

The following deformations of the blood cells are those most likely to be encountered.

Compressed cells. When the cells are closely packed, as often happens in bone marrow preparations and in thick portions of the film, the pressure may be sufficient to cause a reduction in size of certain cells, which then appear darker in colour. A plasma cell compressed in this way is shown in the top right hand corner of Fig 143 Even cells which are undoubtedly mature may sometimes undergo this type of deformation (Fig 72 f), thus discounting the hypothesis that these artefacts are a special type of stem cell.

Stricture formation. If the nucleus of a mononuclear cell is subjected to pressure from both sides and is unable to escape, a stricture develops, as shown in Fig 233 A. This type of deformation is more common in binuclear cells, as the nuclei are readily forced apart allowing the cytoplasm to contract between them (Fig 238 N). Such artefacts should not be confused with cells in mitosis nor are they a proof of the existence of amitoses

Cytoplasmic projections are occasionally exhibited by all blood corpuscles (Figs 36 E, 39 B, 45 B, 89 A, 135 C, 135 D, 236 K, 236 L) They occur very frequently on megakaryoblasts and megakaryocytes (Figs 47 I, 161 to 165, 167) the cytoplasm of which is soft and easily stretched.

When films are prepared from nests of young blood cells, the cytoplasm connecting the regional monocytes with the surrounding proliferating cells may be pulled out to form projections of the type shown in Figs. 143, 147 B and 179 A

Cytoplasmic fragments (Figs 33 A, 135 C, 135 D, 153 A below, 184, 234 D, 234 E) Detached fragments of cytoplasm are usually round Their origin can only be determined if they are lying in the vicinity of the cell to which they belong or if they contain

specific granulation. If they are blue and ungranulated, *they are* liable to be mistaken for pathological "blue" blood platelets Lymph gland preparations usually contain large numbers of cytoplasmic fragments In the case of the megakaryocytes, the detachment of fragments of cytoplasm is a normal physiological process which takes place within the body.

Nuclear projections (Figs 39 B above, 167, 236 K) These projections consist of chromatin which has escaped from the nucleus and penetrated into the surrounding cytoplasm They occur frequently in cells which have been suspended in citrate solution, and may be exhibited by any species of cell In the megakaryocytes, such artificial nuclear projections may be mistaken for the normal passage of intact nuclear substance into the cytoplasm.

Crushed cells. If a cell is compressed or distorted beyond the limits of its elasticity, the first effect is that it is merely pressed flat. The nucleus becomes thin, but still retains its coherent chromatin structure, and if nucleoli are present they become clearly visible The cytoplasm still adheres to the nucleus but is often torn and forms projections of various shapes This type of deformation may be seen in the young stages of development of all the blood cells (Figs 3 right, 46 D centre, 235, 236) with the exception of the lymphocytes The cytoplasm of the latter escapes on the slightest injury and dissolves in the surrounding plasma (Fig. 234) In flattened mitoses, the crushed chromosomes appear pale and considerably broader and longer than intact chromosomes. Flattened blood cells have been regarded as "haemohistioblasts", and are known as "Ferrata cells" after the haematologist who first described them The term **"Ferrata stages of crushed cells"** is more exact. The correctness of this description is illustrated by the occasional finding of a binuclear cell ("twinning deformity") in which one nucleus and the neighbouring cytoplasm have been flattened to a Farrata stage while the remainder of the cytoplasm and the other nucleus have remained intact This is a rare finding, but very instructive

If the cells are subjected to a greater degree of trauma, they are finally crushed or torn The detached cytoplasm may be still visible in the form of droplets or may become dispersed in the surrounding blood plasma. Cells with round nuclei give rise *to round* patches known as **"Gumprecht's shadows"** or **"Gumprecht's bodies"** (Figs. 3, 38 C, 47 G, 53, 55 g, 67, 88, 92, 144 B, *144 C, 156 A, 190, 234 F)* It would be better to term them **"Gumprecht** ***stages of crushed cells"***. The transition from Ferrata stages to Gumprecht stages is particularly easy to recognize in cells in which one half of the nucleus has been less severely damaged than the other (Fig 236 L above) The granules of crushed cells remain undamaged, especially in the case of the eosinophils, and may be seen lying near the nuclear shadow. As already pointed out, the lymphocytes are crushed directly to Gumprecht shadows without passing through a Ferrata stage (Fig 234).

Crushed nuclei of segmented leucocytes form several small shadows instead of a single large one (Fig 233 B) The nuclei of crushed monocytes usually contract and adopt a typical boomerang form (Fig 233 C) The chromatin of such nuclei is consequently more compact than that of crushed juvenile forms of neutrophils.

Pseudophagocytosis. If a cell is superimposed on one of a different size, the smaller cell may appear to have been ingested by the larger, especially if the difference in size is very considerable, as in the case of a megakaryocyte and another leucocyte The smaller cell, which may be either above or below the larger, is usually surrounded by a halo due to shrinkage on drying. Such haloes should not be confused with vacuoles resulting from partial digestion. They are, in fact, particularly characteristic of pseudophagocytosis, and may even be seen surrounding blood platelets superimposed on normocytes (Fig 173) The apparently ingested cells show no evidence of degeneration.

Another type of pseudophagocytosis, which occurs within the body, is exhibited by the megakaryocytes of the bone marrow These sometimes trap smaller blood cells in the deep indentations in their cytoplasm. The imprisoned cells may even disintegrate, but are not consumed (see p 67)

Scratched cells. Cells are very liable to be scratched if stained preparations are cleaned with a cloth or with cotton wool on which oil has been allowed to dry. The scratches usually appear blue on violet or red parts of the cell (Fig 195)

VIII. Soiling of Blood and Bone Marrow Films

Figs 220 to 226, and other illustrations.

The soiling of blood and bone marrow films by the presence of foreign cells, has already been discussed on page 19

The following are further possible sources of error when examining preparations.

Charcoal particles and pollen. If films are exposed to a smoky atmosphere, they may become contaminated with charcoal particles These may be easily recognized by their colour and by their sharp corners (Fig 131 A below) Similar remarks apply to pollen.

Precipitates of stain (Figs 16, 152 B) When a solution of Giemsa stain is diluted with water, a thin, iridescent film forms on the surface. This film should not be allowed to come into contact with the preparation side of the slide, as it attaches itself firmly and cannot be removed. Small particles of precipitate are very liable to be deposited on the borders of the central depressions in hypochromic normocytes (Fig 16) Larger precipitates (Fig 152 B) may cover whole areas of the preparation, either as fine granules, or as continuous violet streaks. Small patches of stain should not be confused with blood platelets.

Glass defects. (Fig 139 A) Scratches in the glass are very frequent and are unlikely to cause difficulty. On the other hand, air bubbles in slides manufactured from poor quality glass may be very troublesome. If they are situated on the surface, they may be open to the air, forming round depressions or grooves which retain staining solution and become coloured red or violet. Small bubbles may thus be mistaken for scattered granules, while larger ones covered by erythrocytes may be confused with Howell-Jolly bodies or nuclei. Such defects should always be suspected if the portion of the slide beyond the film contains air bubbles. Since the Second World War, increased numbers of defective slides have appeared on the market. As they render valuable portions of the film unsuitable for examination and photographic reproduction, their use should be avoided.

IX. Blood Parasites

Figs. 239 to 256.

Parasites which are likely to be found in films prepared from the blood and haemopoietic organs include spirochaetes, leptospiras, malarial parasites, trypanosomes, leishmanias, microfilarias, bartonellas and blastomycetes The forms considered here are mainly those occurring in the blood, and only their morphological characteristics, as seen in the appropriate illustrations, will be described.

SPIROCHAETES AND LEPTOSPIRAS

Fig. 239.

The most important representatives of this group found in man are: *Spirochaetes recurrentis*, *Spirochaetes duttoni* and *Leptospira icterohaemorrhagica.*

Spirochaetes recurrentis (Borrelia recurrentis) (Fig. 239). This is the causative agent of **European relapsing fever** and is transmitted by the clothes lice *(Pediculus vestimenti)*. Its length is 10—20 μ. The spirals are very fine, and measure 2—3 μ in length In the natural state, they are very regular, but when seen in blood films, they are greatly distorted.

Spirochaetes duttoni (Borrelia duttoni). This is the causative agent of **African or tick relapsing fever,** and is very similar to *Spirochaetes recurrentis.* Morphologically, the two varieties are indistinguishable. The vectors are ticks *(Ornithodorus moubata)*

Leptospira icterohaemorrhagica. The causative agent of **Weil's disease (swamp fever, icterus febrilis).** Transmitted to man by water polluted by the urine of the water rat *(Rattus norvegicus)*. The parasite can only be found in the blood during the first few days of the disease, and is best seen using dark ground illumination. It consists of a very fine filament, 5 to 25 μ in length, with very close, regular spirals which give it the appearance of a miniature rope The ends are pointed and often curved over in the form of a hook The parasite is highly motile.

MALARIAL PARASITES (PLASMODIA)

Figs 240 to 247.

The malarial plasmodia are protozoa and belong to the class of *Sporozoa.*

Three species of plasmodia are found in man· *Plasmodium falciparum, Plasmodium malariae* and *Plasmodium vivax*

The parasites are transmitted to man by certain varieties of mosquitoes *(Anopheles)* Both sexual and asexual forms are found in human blood, but the former are incapable of reproduction while in the human body. The asexual forms are known as schizonts and reproduce by schizogony. The young schizont contains a single, brilliant red nucleus, situated in a vacuole surrounded by a blue ring. The mature schizont (segmenter) contains several nuclei and, after bursting the red cell which it has invaded, breaks up into a number of free merozoites These invade fresh erythrocytes and the cycle begins again. The sexual forms produced in the blood of warm-blooded animals are known as gametocytes, the males being microgametocytes and the females macrogametocytes They remain inactive until the blood containing them is ingested by a female mosquito They then mate in the intestine of the insect and produce numerous sporozoites (falciform bodies) If a bite is inflicted by the infected mosquito the sporozoites pass into the blood and cause a new infection The different species of plasmodia may be distinguished from one another by a number of characteristic features

Plasmodium falciparum, Figs 240 to 242 Causes **estivo-autumnal malaria.**

The young schizont has the form of a very small ring, the diameter of which is approximately one fifth of that of an erythrocyte, and little more than half that of the corresponding forms of the other plasmodia The segmenters (Figs 240 F, 242 B), which are only rarely found in the peripheral blood, contain about 16 nuclei, these later develop into the young merozoites The sexual forms (Figs 241, 242 C) have a very characteristic

appearance. They are elongated, being longer than the diameter of an erythrocyte, and often crescent-shaped (crescent forms). The female gametocyte (Figs. 241 A to 241 C) consists of blue cytoplasm, in the centre of which is the small nucleus surrounded by pigment. In the male gametocyte (Figs 241 D, 241 E), the nucleus and pigment occupy almost the entire cytoplasm, which has a reddish-blue colour. The parasites are only slightly motile. They do not cause an increase in size of the erythrocytes which they invade, but if the red cells are deeply stained, they sometimes exhibit a coarse, irregular, dirty greyish-violet granulation, known as **Maurer's dots** (Fig 240 D). Monocytes containing stored pigment from destroyed parasites may often be found in the blood and bone marrow (Figs 115 A, 115 B).

Plasmodium malariae, Figs 243, 244. Causes **quartan malaria.**
The young asexual forms are ring-shaped and of the same size as the corresponding forms of *Plasmodium vivax*, from which they are indistinguishable. Their diameter is approximately one third that of an erythrocyte (Fig 244 C). They soon elongate to the so-called band forms, characteristic of this species of parasite (Figs 243 A to 243 D). The adult schizonts (Fig 243 E) entirely fill the invaded erythrocytes. They usually contain only 8 nuclei (merozoites) surrounding a central mass of pigment ("daisy forms"). The sexual forms (Figs. 244 A, 244 B) are round, like those of tertian malaria, but not as large. The parasites are less motile than *Plasmodium vivax*. As in the case of falciparum, there is no increase in size of the invaded erythrocytes.

Plasmodium vivax, Figs 245 to 247. Causes **tertian malaria.**
Plasmodium vivax is the most widely distributed malarial parasite. It is the only species found in man which causes a marked increase in size and loss of colour of the invaded erythrocytes. The young schizont (Figs 245 A, 245 B), has a ring shape with a diameter about one third that of an erythrocyte. In dried preparations, the older schizonts adopt a great variety of forms, as this parasite, in contrast to the other two, is highly motile (hence the name "vivax") and frequently dries in bizarre positions. The adult schizont (segmenter, Figs 245 D, 245 E) is larger than a normal erythrocyte and contains about 20 nuclei (merozoites). Its pigment usually collects in the centre. The young gametocytes have no vacuoles (Fig 246 A) and are more compact than the asexual forms. The adult male gametocyte (Figs. 246 D, 246 E) is approximately the same size as a normal erythrocyte, but the female (Figs 246 B, 246 C) is somewhat larger. Some of the invaded erythrocytes exhibit a very characteristic brilliant red granulation, known as **Schüffner's dots** (Figs 245 C, 246 B, 247 B).

Plasmodium ovale is a variety of *Plasmodium vivax* which is of identical appearance but causes the invaded erythrocytes to assume an oval form. A further difference is that the schizont divides into only about 12 merozoites.

PRINCIPAL MORPHOLOGICAL CHARACTERISTICS OF THE THREE SPECIES OF PLASMODIUM CAUSING HUMAN MALARIA

Plasmodium falciparum (estivo-autumnal malaria). Young forms, small and ring-shaped, gametocytes, crescent-shaped. Erythrocytes of normal size, occasionally contain Maurer's dots.

Plasmodium malariae (quartan malaria). Schizonts, band-shaped. Erythrocytes of normal size.

Plasmodium vivax (tertian malaria). Erythrocytes greatly increased in size, often contain Schüffner's dots.
In thick drop preparations, differentiation is more difficult than in films, as the plasmodia, like the blood corpuscles, undergo marked shrinkage.

TRYPANOSOMES

Fig 248

The trypanosomes are protozoa and belong to the class of Flagellates. Four species of trypanosome are found in man: *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Trypanosoma cruzi* and *Trypanosoma rangeli*. The forms found in the blood have a narrow body with a central nucleus and are motile. The flagellum is attached to the forward end of the parasite and extends backwards along its body forming the outer edge of an undulating membrane. It ends in the kinetoplast which is situated near the tail end of the body and stains red, like the nucleus. The trypanosomes reproduce by longitudinal division.

Trypanosoma gambiense (Figs 248 A, 248 B) and **Trypanosoma rhodesiense.** These are the causative agents of **sleeping sickness**, a kind of meningo-encephalitis. The two species, both of which are found only in Africa, are almost indistinguishable from one another. Clinically, however, there is a clear distinction between the two infections, the gambiense infection having a chronic course, while rhodesiense sleeping sickness is acute. The parasites vary greatly in size, the slender forms being on the average, 26 μ long and 1 to 3 μ thick. They are transmitted by the tsetse fly *(Glossina)*.

Trypanosoma cruzi (Schizotrypanum cruzi), Fig 248 C. This parasite is the causative agent of **Chagas' disease**, an infection characterized by anaemia, oedema and enlargement of the lymph glands, spleen and liver. No symptoms attributable to affection of the central nervous system are observed. The parasite is found only in Middle and South America and is transmitted by the assassin bug *(Triatoma)*. Its length varies between 15 and 20 μ. It is shorter than the gambiense and rhodesiense parasites but has a considerably longer flagellum and a clearly visible kinetoplast.

LEISHMANIAS

Fig 249.

The **Leishmania** are protozoa and, like the trypanosomes, belong to the class of blood flagellates. There are three species of *Leishmania* found in man· *Leishmania donovani*, *Leishmania tropica* and *Leishmania brasiliensis*. Morphologically, these are indistinguishable from one another. They are transmitted to man by small flies, species of *Phlebotomus* The parasites are round or oval, 2—6 μ in length and have a nucleus and a kinetoplast but no external flagellum They are found only intracellularly within the monocytes of the blood and internal organs (these cells are identical with the "reticulum cells" and with certain "endothelial cells") The parasites may multiply to such an extent that they completely fill the cells (Fig 249 A) which are then very easily ruptured Consequently, films very often contain free parasites lying in groups around the naked nuclei of the cells (Fig 249 B).

Leishmania donovani, Fig 249 This is the causative agent of **intestinal leishmaniasis** or *tropical splenomegaly*. In India, this disease is known as Kala-azar, and in Mediterranean countries, where it mainly affects children, as infantile splenomegaly. The parasites are found principally in the monocytes of the spleen, the liver, the bone marrow, the lymph glands, the intestines and the skin In feverish attacks, they may also appear in the monocytes of the peripheral blood. The diagnosis may often be confirmed by the presence of the parasites in the sternal marrow.

Leishmania tropica. Causes **oriental boil** (Aleppo boil, Biskra button, etc) This parasite produces centres of infection beneath the skin, with the appearance of boil-like eruptions which later develop into ulcers

Leishmania brasiliensis. Causes **South American leishmaniasis** or **cutaneous leishmaniasis.** This species produces ulcers direct, without the preceding appearance of boils They frequently appear upon the face and may also spread to the mucosa.

The last two of these *Leishmania* remain localized in the skin, penetrating, at the most, to the regional lymph glands Consequently, the parasites may sometimes be found in lymph gland puncture preparations, and therefore also have a certain haematological interest.

FILARIAE

Figs 250 to 253.

The filariae are thread worms, belonging to the class *Nematodes*. Their intermediate hosts are certain species of gnats and flies When transmitted to man, the parasites settle in one particular organ of the body, which varies according to the variety of filaria. There they produce larvae, known as **microfilariae,** which, in certain species, pass into the blood and may be readily detected in thick drop preparations Only the microfilariae are of haematological interest. They have an elongated body, made up of a chain of so-called body cells, containing clearly visible, dark nuclei. Taken in conjunction with the presence or absence of an integument (sheath), the number and arrangement of the nuclei enable the species of filaria to be determined. Approximately a dozen different filariae are found in man, but only those species whose larvae pass into the blood are discussed here

1. FILARIAE HAVING SHEATHED LARVAE (MICROFILARIAE)

Wuchereria bancrofti. Causes **Filariasis bancrofti.** This filaria resides in the lymphatic vessels As it multiplies, the vessels become blocked and this, in conjunction with infections, leads to elephantiasis. The microfilaria (Figs 251, 253 B) is about 280 μ long and 7 μ thick. It is surrounded by a sheath and the nuclei of the body cells do not reach to the end of the tail It is usually found in the blood only at night (hence the old name *Filaria nocturna)*

Loa loa. Causes **loiasis, calabar swellings and ocular filariasis.** The loa worm roams in the subcutaneous tissues and gives rise to fleeting cutaneous swellings ("fugitive swellings"), but not to elephantiasis. It often penetrates into the anterior portions of the eye ("eye worm") The microfilaria (Figs 250 left, 253 A) is 250 to 300 μ long and 6 to 8 μ thick, so that it is about the same size as that of *Wuchereria bancrofti*. It also has a sheath, but, in this case, the nuclei reach to the end of the tail It is found in the blood only during the day *(Filaria diurna).*

Wuchereria malayi. Causes **Filariasis malayi** or **Brug's filariasis.** The parasite resides in the lymphatic vessels and provokes elephantiasis The microfilaria (Figs 252, 253 C) is surrounded by a sheath and is found in the blood only at night It differs in appearance from *Microfilaria bancrofti* by the presence of two nuclei in the end of the tail

2 FILARIAE HAVING UNSHEATHED LARVAE (MICROFILARIAE)

In some regions, more than 90 per cent of the population are infested with the parasites Infestation does not, however, produce any tangible symptoms of disease

Dipetalonema (Acanthocheilonema) perstans. The parasite is found in the serous cavities of the body The microfilaria (Fig 250 right) is about 200 μ long and 4 μ thick, so that it is somewhat shorter than the species previously discussed, and only half as thick. Moreover, it has no sheath The tail is short and contains nuclei right to the point. The microfilariae may be found in the blood throughout the 24 hours

Mansonella ozzardi. The only morphological difference between the microfilaria of this species and that of *Dipetalonema perstans* is that it has a pointed tail which contains no nuclei

BARTONELLAS

Fig. 254

The position to be assigned to the *Bartonella* in the system of micro-organisms has not yet been settled. In certain respects, they resemble the *Grahamella* and *Rickettsiae*.

Bartonella bacilliformis (Fig. 254) is the only pathogenic species of *Bartonella* found in man. In its most usual form the organism is a small, delicate rod, 1 to 2 μ in length and 0.2 to 0.5 μ in width; more rarely it has a spherical form, with a diameter of 0.3 to 1.0 μ. By Giemsa's method the organism stains purple. The *Bartonella* invade the erythrocytes and monocytes and often adopt a V- or Y-shape. They are the causative agent of **Carrión's disease.** The first stage, known as **Oroya fever,** runs an acute course accompanied by a high temperature. If untreated, death occurs within 2 to 3 weeks in 20 to 40 % of the cases. Examination of the blood shows that the erythrocytes are packed with large numbers of organisms, thus confirming the diagnosis. The feverish stage is followed by a chronic stage, usually called **verruga peruana,** which sets in 3 to 4 months after the appearance of the first symptoms. Verruga peruana is characterized by the appearance of nodular and wart like eruptions on the skin. The parasites are transmitted to man by certain sand flies (species of *Phlebotomus*), but the infection cannot be transferred from one human being to another. Carrión's disease is widespread in certain parts of the Andes between a height of 2,500 and 10,000 feet. This region includes not only Peru but also Ecuador and Columbia. The disease is named after the Peruvian physician Carrión, who infected himself with the disease in 1885 and demonstrated the common cause of the two forms. Unfortunately, the experiment had a fatal outcome. The causative organism was discovered in 1909 by another Peruvian physican, Barton.

BLASTOMYCETES

Figs. 255 and 256

The blastomycetes belong to the yeast family and reproduce by budding.

Paracoccidioides brasiliensis (Blastomyces brasiliensis, Zymonema brasiliensis). This is the causative organism of **Blastomycosis brasiliensis** or **South American blastomycosis,** and is the only representative of the genus which is of haematological interest. It is generally found in the lymph glands and its detection in lymph gland punctate offers a useful means of confirming the diagnosis in doubtful cases. The organism is spherical, double-walled, and varies in size up to 20 μ in diameter. It is chromophobic and strongly refracting to light. In the lymph glands, the organisms either occupy giant cells (Fig. 255), which are apparently highly polyploid monocytes (cf. Fig. 130), or form large groups in the necrotic tissue (Fig. 256). Sometimes they are surrounded by a ring of neutrophils or eosinophils. The disease is widely distributed in South America, especially in Brazil. The infection usually starts in the buccal cavity.

PART THREE

Illustrated Section with Explanatory Notes

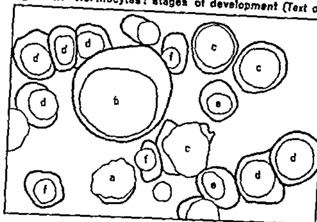
Plate 1

Erythrocytes: Normocytes and Megalocytes

Stages of development and differences in size and structure

Figure 1. Normocytes: stages of development (Text [illegible] pronormoblasts (macronormoblasts, macroblasts [illegible] are: In A, *below*, a polychromatic normoblast, a [illegible] ix macro- [illegible]
Bone marrow films from children with secondary anaemia and cells of normal appearance Pappenheim staining (By courtesy [illegible] present of the Children's Hospital, Basle)

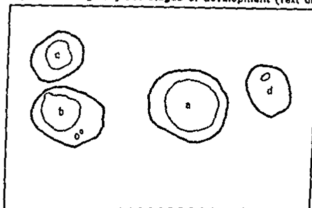
Figure 2. Normocytes: stages of development (Text on pp 44, 45).



a) Pronormoblast (damaged)
b) Macronormoblast (*very large specimen*)
c) *Basophilic* normoblasts
d) Polychromatic normoblasts
e) Oxyphilic normoblasts *with nuclear structure*
f) Oxyphilic normoblasts with *structureless* nuclei
Also present are deformed *normocytes*
Anaemia *in a child* with normoblasts *of* normal appearance. Bone marrow film Pappenheim staining

Figure 3. Megalocytes: stages of development (*Text on p 45*) Centre: two promegaloblasts *Left*: two basophilic megaloblasts *Bottom right*: oxyphilic megaloblast with structureless nucleus. In addition, *on the extreme right* is a flattened megaloblast *Below*: crushed, denuded nucleus ("nuclear shadow") of a mature megaloblast. *So-called* "megaloblastic marrow", characteristic of untreated pernicious anaemia.
Pernicious anaemia before treatment. Man aged 55 Bone marrow film *from the same case as in* Figures 4, 25 C, 27–29, 37–40 C, 40 E, 41, 86, 101 B, 163 G, 178 A, 231 A, 236 K. Giemsa staining.

Figure 4. Megalocytes: stages of development (Text on p. 45).



a) Basophilic megaloblast
b) Polychromatic megaloblast with two *Howell-Jolly* bodies
c) Oxyphilic megaloblast with *nuclear structure*
d) Megalocyte with Howell-Jolly body Also *present* are megalocytes and normocytes Poikilocytosis and anisocytosis
Pernicious anaemia before *treatment* Bone *marrow film*. Giemsa staining (From the same case as Figure 3, *etc*)

Figure 5. Normocytes (Text on p 45) Healthy individual. Blood film Giemsa staining

Figure 6. Megalocytes (Text on p. 45). *Some of the megalocytes are slightly elliptical*
Pernicious anaemia before treatment. Woman aged 55. Blood film. Giemsa staining (From the same case as Figures 13 14 and 40 D).

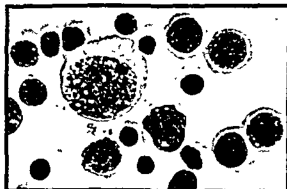
Figures 1, 2 and 3 show that macropronormoblasts may attain the size of promegaloblasts. The distinguishing feature is the noticeably coarser structure of the nucleus in the macropronormoblasts. Normocytes never reach the size of megalocytes.

Magnification 1 1200

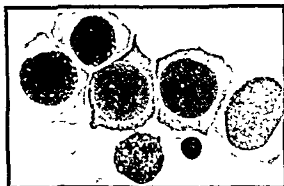


1 A. B C

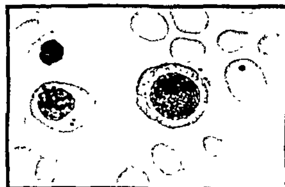
Normocytes stages of development
Pronormoblasts and macro pronormoblasts (macroblasts)



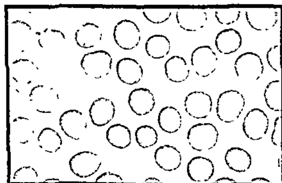
2 Normocytes all nucleated stages of development



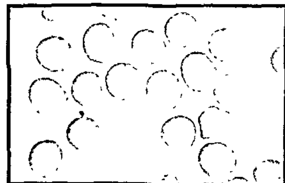
3 Megalocytes stages of development
Two promegaloblasts two basophilic and an oxyphilic megaloblast in bone marrow in untreated pernicious anaemia



4. Megalocytes stages of development
A basophilic, a polychromatic and an oxyphilic megaloblast, megalocyte with Howell Jolly bodies

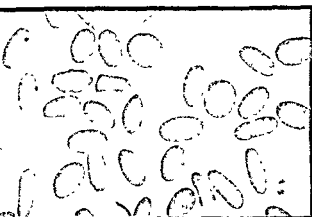


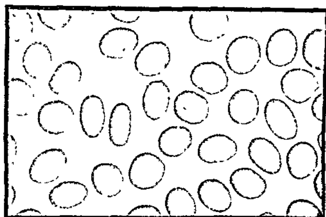
5 Normocytes

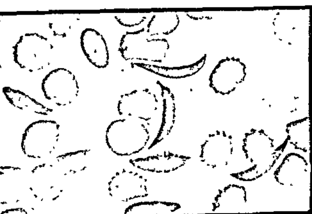


6 Megalocytes

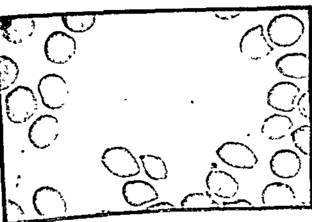
gnification 1 1200


. Elliptocytosis, complete carrier


8 Elliptocytosis, partial carrier


). Drepanocytes


10. Microspherocytes


11. Half moon bodies

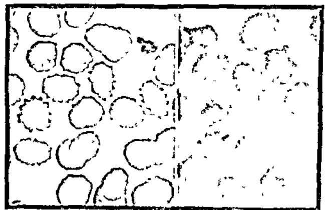

12. A Crenated normocytes B Burr cells

Plate 2

Erythrocytes: Normocytes

Cell forms in anomalies; reactive and artificial morphological changes

Figure 7. Complete carriers of elliptocytosis (Text on p 46) All the normocytes are elliptical (oval)
Blood film Pappenheim staining (By courtesy of Dr S J Leitner, Leysin)

Figure 8. Partial carriers of elliptocytosis (Text on p 46) Of the normocytes completely visible, approximately 50% have a more or less pronounced elliptical form.
Blood film Giemsa staining

Figure 9 Drepanocytosis (sickle cell anomaly) (Text on p 46) Normocytes, some showing drepanocytosis The sickle cells are more intensely stained than those which are not deformed
The presence of some crenated red blood corpuscles indicates that the formation of sickle cells is not equivalent to the production of crenated forms
Drepanocytic (sickle cell) anaemia Negro boy Blood film Wright's staining (By courtesy of Prof L. R. Limarzi, Chicago)

Figure 10 Microspherocytes (Text on p 46) Microspherocytes of different sizes and with very deep coloration due to the thickness of the cells
Congenital haemolytic jaundice during the crisis Blood film Giemsa staining
Notice the small diameter of these cells in comparison with the normocytes in Figure 5 and the megalocytes in Figure 6.

Figure 11. Half-moon (crescent) bodies (Text on p 47) The crescent bodies are the large, pale elements They contain no haemoglobin and stain pure red
Hypochromic anaemia Blood film. Pappenheim staining

Figure 12. A. Crenated red blood corpuscles (Text on p 46) Some of the normocytes are crenated These forms are artefacts: when films are allowed to dry slowly, cell fluid passes from the normocytes into the surrounding plasma which becomes increasingly hypertonic as drying proceeds
Healthy person Giemsa staining Blood film
B. "Burr-Cells" (Text on p 47) Five of the normocytes exhibit a peculiar, bizarre deformation, giving them a resemblance to the clinging burrs of certain plants, particularly the cells at the top left and in the centre (Schwartz and Motto [1]) They are a kind of poikilocyte, cf Figure 14
Tuberculosis treated with thiosemicarbazone Blood film Pappenheim staining (By courtesy of the University Medical Clinic, Zurich).

Plate 3

Erythrocytes: Normocytes and Megalocytes

Changes in size and structure; overproduction of immature, non-nucleated elements

Figure 13. Anisocytosis (Text on p 47). The existence of marked differences in size among the erythrocytes is known as anisocytosis This figure shows megalocytes, normocytes and microcytes lying side by side.
Pernicious anaemia before treatment Blood film. Giemsa staining

Figure 14. Poikilocytosis (Text on p 47) Poikilocytes are irregularly deformed erythrocytes. Often they are only fragments of ruptured erythrocytes The pear-shaped form, like that in the top left hand corner, is frequent. Anisocytosis is also present
From the same film as Figure 13.

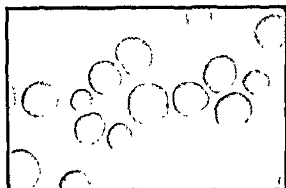
Figure 15. A. Target cells (Text on p 46) Three target cells. An anulocyte and a drepanocyte are also present.
Drepanocytosis in a negro boy. Blood film From the same preparation as Figure 9
B. Anulocytes (Text on p 47) Normocytes with central depression due to lack of haemoglobin; so-called anulocytes or pessary forms
Hypochromic anaemia in leukaemia. Blood film Giemsa staining

Figure 16. Central acidophilic stippling in anulocytes (Text on p 47). Anulocytes as in Figure 15 B. but with red stippling around the central depression.
Hypochromic anaemia. Blood film Pappenheim staining

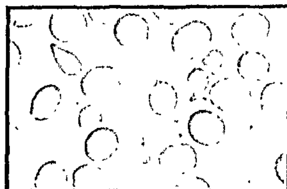
Figure 17. A. Polychromasia (Text on p 47) Two bluish-red (polychromatic) normocytes Anulocytes are also present.
Secondary anaemia in leukaemia. Blood film. Giemsa staining From the same case as Figure 15 B.
B. Basophilic stippling (Text on p. 47) Two normocytes with blue, so-called basophilic, stippling
Hypochromic anaemia. Blood film Giemsa staining

Figure 18. Proerythrocytes (reticulocytes, text on pp 44, 47) Proerythrocytes or reticulocytes are elements which reveal basophilic granules and filaments (substantia granulofilamentosa, reticular substance) on supravital staining by Wolfer's method; with Hirschfeld's haemolytic staining they exhibit a felt-like flocculation The proerythrocytes are the normal, non-nucleated precursors of the normocytes; as a rule, therefore, they are somewhat larger than mature normocytes.
Cases of hypochromic anaemia Blood films
Left: Wolfer's stain (see p 27, staining method d)
Right: Hirschfeld's stain (see p 27, staining method e)

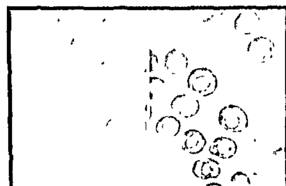
Magnification 1:1200



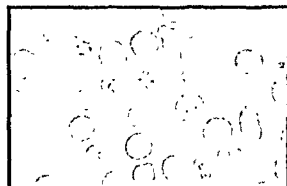
13. Anisocytosis



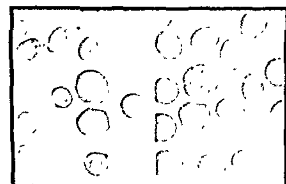
14. Poikilocytosis



15. A Target cells — B Anulocytes



16. Central deposits of stain in anulocytes



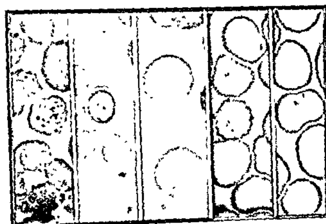
17 A Polychromasia — B Basophilic stippling



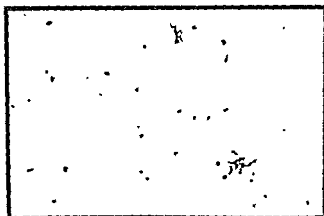
18. A — B *Proerythrocytes (reticulocytes)*

Wolfer's vital staining — *Direct field's stain*

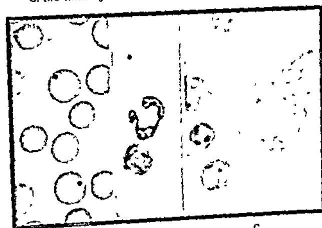
Magnification 1:1200



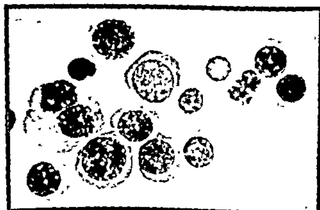
19. Normocytes
A. containing nuclear dust, B containing a Cabot's ring, C. two twinning deformities, D. and E. siderocytes



20. Heinz-Ehrlich bodies



21. Normocytes containing Howell-Jolly bodies
A Giemsa staining, B Feulgen reaction, C Feulgen-positive nucleus for comparison



22. Entrance of normoblasts into blood stream in haemolytic disease of the new born



23. Toxic basophilic stippling and normoblasts in blood



Plate 4

Erythrocytes: Normocytes and Megalocytes

Inclusion bodies; entrance of nucleated stages into blood stream

Figure 19 Special forms of normocytes (Text on pp 43, 47, 48) A. Normocyte containing small particles of nuclear dust (chromatin dust) B Normocyte with Cabot's ring C Two tetraploid normocytes (normocyte twins), mature stages of mononuclear or binuclear tetraploid normoblasts D and E. So-called "siderocytes", normocytes containing particles which give a positive Prussian blue reaction for iron.

A. Healthy individual B Syphilis in a child. C. Haemolytic disease of the new-born. D and E. Atrophy or fibrosis of the spleen A. Bone marrow film B to E. Blood films. A. and B. Pappenheim staining C Giemsa staining D. and E. Grüneberg's Prussian blue reaction (p. 31, staining method q).

Figure 20 Heinz-Ehrlich bodies (Text on p 18). Heinz-Ehrlich bodies are small blue globules which are found in the cytoplasm of the normocytes They usually occur singly

Two proerythrocytes (reticulocytes), containing a violet-coloured network, are also present.

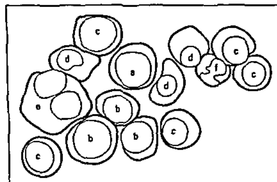
Patient under sulphonamide therapy Blood film Nile blue sulphate staining (By courtesy of the University Medical Clinic, Zurich)

Figure 21. Howell-Jolly bodies (Text on pp 47, 48) A. Giemsa-staining Two of the normocytes contain Howell-Jolly bodies The absence of a halo surrounding the Howell-Jolly bodies, and their homogeneous, dark appearance distinguish them from superimposed blood platelets (see Figure 173)

B and C Feulgen reaction (p 31, staining method p) The reddish-violet colour is given only by true nuclear substance (thymonucleic acid) In B above is a normocyte containing a Feulgen-positive Howell-Jolly body, which indicates that these particles also consist of nuclear substance Below, for comparison, are a segmented neutrophil and a lymphocyte, while C. shows some plasma cells and a megakaryocyte The nuclei only can be seen as they are always Feulgen positive; the cytoplasm is always negative and therefore remains invisible. The blood platelets, like the cytoplasm of the megakaryocytes from which they are derived, also fail to give the Feulgen reaction [2, 3] The erythrocytes show only their own yellowish coloration.

A. and B Condition following splenectomy C. Plasmocytoma Bone marrow film

Figure 22. Entrance of normoblasts into blood stream in haemolytic disease of the new-born (Text on p 48)



a) Pronormoblast.
b) Basophilic normoblasts.
c) Polychromatic normoblasts.
d) Oxyphilic normoblast with nuclear structure.
e) Twinning deformity of a basophilic normoblast.
f) Damaged normoblast with ruptured nucleus

Rhesus-positive new-born with haemolytic disease (erythroblastosis foetalis or neonatorum) due to sensitization of the rhesus-negative mother by the Rh factor Blood film Pappenheim staining

Figure 23. Toxic basophilic stippling and normoblasts in blood (Text on pp 47, 48) A and B Two macrocytes with pronounced basophilic ("toxic") stippling; in the cell in B. a fragment of a Cabot ring may also be seen (for a complete ring, see Figure 19 B). C. and D. Oxyphilic normoblasts with basophilic stippling, the cell in C. contains a rosette-shaped nucleus and below is an elliptical megalocyte E. Polychromatophilic normoblast.

Pernicious anaemia before treatment. Blood film Giemsa staining

Figure 24. Megaloblasts in blood (Text on p. 48) Megaloblasts in various stages of development.

Pernicious anaemia without treatment (before the introduction of liver therapy), prior to death Blood film *Giemsa staining*

Plate 5

Erythrocytes: Normocytes and Megalocytes

Immature forms in pernicious anaemia and elliptocytosis

Figure 25: Megalocytes in an embryo and in a case of pernicious anaemia (Text on p. 43) *In A. and B. and in C. above are three promegaloblasts.* The fine nuclear structure is very similar in all three cells The size of the cell and the size of the nucleus relative to that of the cytoplasm are greater in C. and the staining characteristics are more accentuated. *In the embryo* megalocytosis is *a physiological condition, whereas in patients* with pernicious anaemia it is pathological. *The qualitative differences between the megaloblasts in C. and normal megaloblasts* are apparently due merely to the *severe nature of the systemic disease, i.e. they are secondary changes* Below are four oxyphilic megaloblasts with structureless *nuclei. They are similar in every respect.* The megaloblast in C. below *contains Howell-Jolly bodies, which probably derive from aberrant chromosomes. The cells do not give a peroxidase reaction.*

A. and B from an embryo of about three months. Umbilical blood film Graham-Knoll peroxidase reaction.

C. Untreated pernicious anaemia. Bone marrow film. Giemsa staining.

Figure 26. Oxyphilic megaloblasts and a normoblast in embryo blood (Text on p. 43). *The three megaloblasts are oxyphilic,* the normoblast and the *adjacent normocyte are polychromatic. All the cells* have structureless (disintegrating) *nuclei, indicating that denucleation is imminent.* The difference in size between the megaloblasts and the normoblast is very marked

The same embryo as in Figures 25A. and 25B. Cardiac blood film. Giemsa staining

Figure 27. Nest of normoblasts and a promegaloblast in untreated pernicious anaemia (Text on p. 45) Nest of normoblasts with promegaloblast below. The normoblasts are *considerably smaller than the* promegaloblast. Top left, *a pronormoblast with a more compact nuclear structure than the promegaloblast*

Pernicious anaemia before treatment. Bone marrow film from the same case as Figure 3 Giemsa staining

Figure 28. Polychromatic and oxyphilic normoblasts in the bone marrow in untreated pernicious anaemia (Text on p 45). *Two polychromatic and three oxyphilic normoblasts, one with basophilic stippling* The nucleus of one of the *polychromatic normoblasts has been partly squeezed out during the preparation of the film*, this is an artefact (pseudo-expulsion of the nucleus).

Pernicious anaemia before treatment. Bone marrow film from the same case. Giemsa staining

Figure 29. Normoblasts in the bone marrow in treated pernicious anaemia. *Nest* with three basophilic normoblasts on the left and five polychromatic normoblasts on the right.

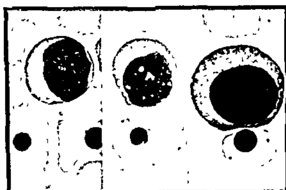
Pernicious anaemia after cure by liver therapy. Bone marrow film from the same case as in the two preceding figures Giemsa staining.

[illegible]

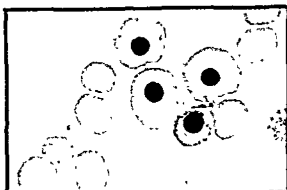
Elliptocytosis. Bone marrow film. Pappenheim staining. [illegible]

Figures 23, 27 and 28 indicate that in untreated pernicious anaemia the blood and the bone marrow also contain normoblasts in addition to megaloblasts.

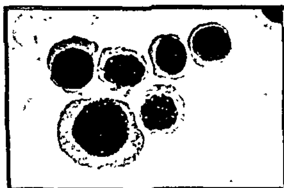
Magnification 1 1200



25. A and B megaloblasts from an embryo in blood,
C megaloblasts in pernicious anaemia in bone marrow



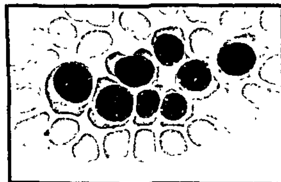
26 Oxyphilic megaloblasts and a normoblast in blood from an embryo



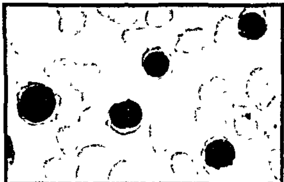
27. Nest of normoblasts and a promegaloblast in untreated pernicious anaemia (bone marrow)



28 Polychromatic and oxyphilic normoblasts in untreated pernicious anaemia (bone marrow)

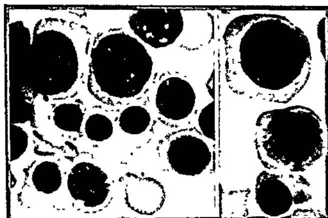


29. Normoblasts in bone marrow in treated pernicious anaemia.



30 Round normoblasts in bone marrow in elliptocytosis

Magnification 1:1200



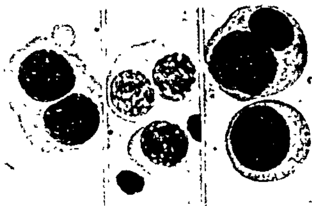
31. A B

Formation of haemoglobin in erythrocytes Detection by the Lepehne reaction A in normoblasts, B in megaloblasts



32. A B C.

Mitosis of normoblasts A and B prophase, metaphase, telophase C twinning deformity with dissociated karyokinesis upper nucleus in prophase, lower in interphase



3. A B C

Tetraploid normoblasts with two diploid nuclei (twinning deformities) In C there is dissociated necrobiosis above, living nucleus, below, dead (necrobiotic) nucleus



34. A B

Highly polyploid giant pronormoblasts

A Multipolar mitosis with numerous chromosomes of normal size, B polynuclear interphase



35 C D

Highly polyploid giant pronormoblasts

C metaphase with giant chromosomes D mononuclear interphase with giant nucleolus



36 E

E mononuclear

slightly compre

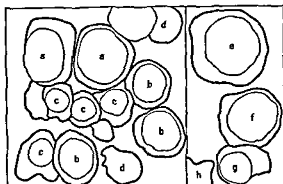
F highly polyploi

Plate 6

Erythrocytes: Normocytes and Megalocytes

Haemoglobin formation; typical and atypical mitoses; deformities

Figure 31. Haemoglobin formation in erythrocytes. Detection by the Lepehne reaction (Text on pp. 23, 44, 45, staining method g, Table 29). The stages containing haemoglobin have olive-green cytoplasm



A. Normoblasts
a) pronormoblasts (negative),
b) basophilic normoblasts (positive),
c) oxyphilic normoblasts with nuclear structure (strongly positive),
d) normocytes (strongly positive).
Anaemia in an adult with normoblasts of normal appearance *Bone marrow film*

B. Megaloblasts
e) promegaloblast (negative),
f) basophilic megaloblast (positive),
g) oxyphilic megaloblast (strongly positive),
h) part of a megalocyte (strongly positive).
Untreated pernicious anaemia Bone marrow film.
(By courtesy of Dr K A. Punschel, Arosa)

In neither species of erythrocyte do the blast cells contain the blood pigment haemoglobin The pronormoblasts and promegaloblasts *are therefore colourless like the leucocytes*, and the Lepehne reaction is negative *Only the later*, more mature stages, the basophilic normoblasts and megaloblasts contain haemoglobin

Figure 32. Mitoses of normoblasts (Text on p 45) A. Basophilic normoblast in prophase B Left: polychromatic normoblast in metaphase Right: basophilic normoblast in telophase; the nuclear spindle is clearly visible C. Tetraploid pronormoblast with two diploid nuclei (twinning deformity) and dissociated karyokinesis: the upper nucleus is in prophase, the lower in interphase ("resting nucleus") — The cytoplasm has the pronounced mottled appearance characteristic of mitosis The chromosomes of the normoblasts are less easy to distinguish than those of the megaloblasts, cf Figures 37 to 41 As in all erythrocytes, the Graham-Knoll peroxidase reaction is negative

A and B normal erythropoiesis, C severe disturbance of erythropoiesis Bone marrow film A. and C. Pappenheim staining B Graham-Knoll peroxidase reaction (C by courtesy of *Prof A. Alder, Aarau*)

Figure 33. Tetraploid normoblasts with two diploid nuclei (twinning deformities) (Text on p 23) A. and B above: both cells contain two living nuclei of equal size C Dissociated necrobiosis in the upper cell the lower nucleus is living, the upper nucleus is dead (necrobiotic) Normally the death of the erythrocyte nuclei does not take place until the stage of the oxyphilic erythroblast is reached, here death has occurred much earlier, but only in one of the two nuclei In A. part of the cytoplasm which has escaped *is seen lying near the upper part of the cell* in the form of a droplet (blue pseudo-platelet). B below basophilic normoblast, C below, macronormoblast.

A Anaemia in a child, bone marrow film B Haemolytic disease of the new-born, blood film. C. Pathological erythropoiesis, same patient as Figure 32 C, bone marrow film. (C by courtesy of Prof A. Alder, Aarau)

Figures 34 to 36. Highly polyploid giant pronormoblasts and a highly polyploid normocyte (Text on p 23).

A. Multipolar mitosis with numerous chromosomes of normal size. The chromosomes have divided and the nuclei have doubled at each karyokinesis, as is normally the rule, only the cytoplasm having remained undivided B Polynuclear interphase ("resting nuclei") The nuclei are of various sizes and partly cover one another; they are probably six in number Small nucleoli C. Metaphase with giant chromosomes The giant chromosomes have apparently arisen during the preceding karyokineses as the result of repeated doubling of the chromosomes without division. About 24 chromosomes can be counted. D and E. Giant pronormoblasts in interphase

In D a normocyte is lying on top of the giant cell (pseudophagocytosis) In F. the giant normocyte has been indented by the *pressure of a* polychromatic normoblast.

The intermediate stages from giant pronormoblast to giant normocyte—giant basophilic, polychromatic and oxyphilic normoblasts—can also be observed, but are not shown here

Plate 7

Erythrocytes: Megalocytes

Typical and atypical mitoses and deformed cells

Figures 37 to 41. Mitoses and deformities of megaloblasts (Text on pp. 23, 24, 45, 48) Pernicious anaemia before treatment. Bone marrow films from two cases Giemsa staining Figures 37 to 39, 40 C, 40 E. and 41 are from the same case as Figures 3, etc.; Figure 40 is from the same case as Figure 6.

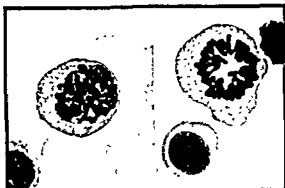
Figures 37 and 38. Typical mitoses of diploid megaloblasts A to C basophilic megaloblasts, D. polychromatic megaloblast A. Prophase (monospirem), B metaphase (monaster), C. anaphase (diaster), D telophase (dispirem) In addition, a polychromatic megaloblast is visible in B. and a *Gumprecht's* shadow of a crushed megaloblast in C. To be noted are the large hof in the monaster of the metaphase B. and the very small hofs in the diaster of the anaphase C.

Figures 39 and 40. *Interphases* ("resting phases") and mitoses of polyploid megaloblasts A. Interphase ("resting phase") of a tetraploid megaloblast with two diploid nuclei. It is the product of an atypical mitosis of a diploid megaloblast, no division of the cytoplasm having taken place after division of the nucleus. B Prophase of such a double cell. Two nuclei in prophase are present side by *side*, forming a double monospirem Part of the upper spirem has been squeezed out and forms a projection. C. Metaphase of a double cell The broad central hofs of the asters indicate that this is a double cell with two monasters and not an anaphase of a mononuclear cell. D Anaphase of a double cell. That this is an anaphase can be recognized from the fact that the central hofs are barely visible. It must also be a double cell because four asters are present A further atypical feature is that the individual asters are connected by chromosome bridges, the forerunners of the nuclear bridges in the subsequent interphase. E. Interphase of a polyploid, oxyphilic megaloblast with five nuclei The two smaller nuclei are probably fragments of a single nucleus and originated from chromosome groups which failed to unite again after the preceding mitosis This is apparently an octoploid cell with three diploid and two hypodiploid *nuclei*. The nuclei are connected by chromatin bridges, a further consequence of the markedly atypical nature of the preceding mitosis The chromatin bridges develop from the chromosome bridges and are not to be interpreted as evidence of amitosis In addition, diploid basophilic megaloblasts can be seen below in B. and C.
Figures 39 and 40 show that multinuclear deformities result from atypical mitoses and not from amitoses.

Figure 41. A Atypical mitosis of a megaloblast with some aberrant chromosomes. *Left*, a megalocyte with three Howell-Jolly bodies. B Polychromatic megaloblast with two nuclei and Howell-Jolly bodies The latter are probably the remains of aberrant chromosomes which are no longer capable of independent existence after karyokinesis and become transformed into the necrobiotic Howell-Jolly bodies.

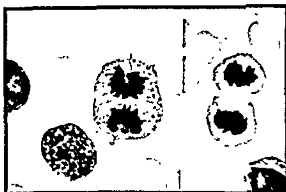
Figure 42. Megaloblasts and a neutrophilobiast (Tables 7, 8, 9, pp 42, 43) Below, two promegaloblasts with typical small-mesh, fine, radial nuclear structure Top right, neutrophiloblast (myeloblast of the neutrophilic series) with large-mesh, convolute nuclear structure In addition, in the top left-hand corner is a basophilic megaloblast, with a mature neutrophilic myelocyte to the right; on the left, below, is part of a staff neutrophil, and in the bottom right-hand corner, part of a monocyte
This figure demonstrates the structural differences between the promegaloblasts and stem cells of one species of leucocyte.
Pernicious anaemia before treatment. Bone marrow film Giemsa staining

Magnification 1 1200



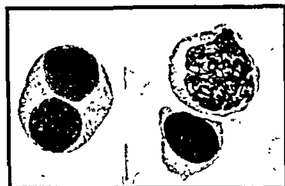
37. A B

Megaloblasts A in prophase B in metaphase



38. C D

C in anaphase D in telophase



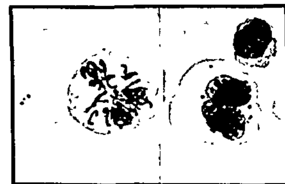
39 A B

Tetraploid megaloblasts with two diploid nuclei (twinning deformities) A in interphase, B in prophase



40 C D E

The same C in metaphase, D in anaphase, E megaloblast with five nuclei



41 A B

Megaloblasts

A atypical mitosis with aberrant chromosomes,

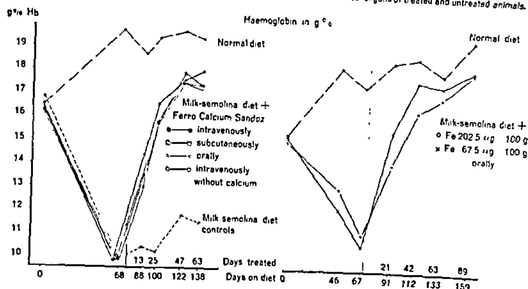
B twinning deformity with Howell-Jolly bodies



42 Megaloblasts and one neutrophiloblast in untreated pernicious anaemia (bone marrow)

Anaemia in rats fed on a diet of cows' milk and semolina: *response to treatment* with **Ferro-Calcium-Sandoz.**

Above: haemoglobin curves. Below: macroscopic Prussian Bleu reaction on various organs of treated and untreated *animals.*



Brain

Liver

Pancreas

Spleen

Kidney

Mammary glands

Lymphatic glands

Peyer's patches

Ovary, fallopian and utes

a	b	c	d	e
Controls		Milk-semolina diet + 68 days' Fe		
Normal diet	Milk semolina diet	intravenously	orally 67.5 μg Fe per 100 g rat day	orally 202.5 μg

Plate 8

Investigation of the therapeutic action of ferrous iron in iron-deficiency anaemia

Problem

1. Study of the effects of a bivalent iron preparation on experimental iron-deficiency anaemia in the laboratory white rat, and comparison of the intravenous, subcutaneous and oral routes of administration.
2. Comparison of the effects of iron given with and without the addition of calcium.

Procedure

Animals

Young female rats about 40 days old were placed singly in containers free from iron. A number of these animals were fed a normal mixed diet and served as **normal diet controls**. The remainder received a special milk diet (milk-semolina diet). This diet has a very low iron content and, given during the growth period, leads to an **iron-deficiency anaemia**. The haemoglobin value of the blood falls and there is a sharp reduction in the iron content of the iron reservoirs. By about the 60th day the anaemia becomes very pronounced. When this point was reached, the animals were divided up into four sub-groups of 15 to 20 animals each, three groups being treated with the preparation to be tested, and the fourth serving as **milk diet controls**.

Preparation tested

Ferro-Calcium-Sandoz is a solution of ferrous lactobionate and calcium gluconolactobionate for intravenous and intramuscular injection in man. The calcium salt is added merely for the purpose of preventing the side effects which are liable to occur on intravenous administration of bivalent iron preparations. Ferro-Calcium-Sandoz was administered to the rats intravenously, subcutaneously and orally. In order to prove that calcium does not inhibit the increase in haemoglobin, one group received ferrous lactobionate intravenously without addition of calcium.

Ferronicum is a well-tolerated preparation of ferrous gluconate in the form of sugar-coated tablets for oral administration in man. Experiments not described here have shown that ferrous gluconate has exactly the same action by mouth as ferrous lactobionate.

Dosage and Method of Administration

Left-hand curve: 67·5 μg Fe/100 g rat every other day intravenously, subcutaneously or orally

Right hand curve: 67·5 μg and 202·5 μg **(triple dose)** Fe/100 g rat every day orally

Sections of organs (in colour): 67·5 μg and 202·5 μg **(triple dose)** Fe/100 g rat every day orally

Assessment of Absorption

Haemoglobin content of the blood: determined in g % (16 g % = 100 corrected Sahli units)

Iron content of the internal organs: estimated by the macroscopic Prussian blue reaction, the intensity of which is proportional to the iron value determined by analysis.

Results

Plate 8, above: haemoglobin curves

The haemoglobin content of the blood is high in animals receiving normal diet, but is greatly diminished when a milk-semolina diet is given. In untreated animals the values remain low; in animals treated with iron they climb rapidly. The curves on the left show that the rise in haemoglobin is approximately the same whether the iron is administered intravenously, subcutaneously or orally, and that it is unaffected by addition of calcium. The curves on the right show that if the oral dose is tripled the rise in haemoglobin takes place more rapidly.

Plate 8, below: sections of organs

Life-size illustrations of portions of different organs taken from one typical animal from each group.

The influence of the diet on the iron content, as determined by the macroscopic Prussian blue reaction, varies according to the organ examined. Thus the iron in the substantia nigra of the brain is entirely retained, and the iron content of the lymphatic glands and the uterus remains almost unchanged. In contrast, the classical iron reservoirs, the liver and the spleen, lose their iron completely.

The liver of animal **a** (normal diet) is dark blue, showing that it contains much iron. In animal **b** (milk-semolina diet) the liver is colourless and therefore contains no iron. The livers of animals **c**, **d** and **e** also fed on milk semolina diet but treated with iron, again contain iron. In animal **c**, treated with iron intravenously, the liver contains as much iron as that of animal **a** on a normal diet. In animal **d**, treated orally for the same period and with the same dose as animal **c**, and in animal **e**, treated with three times this dose, the blue coloration is not so intense, showing that less iron is present.

These experiments demonstrate that, in experimentally produced iron deficiency anaemia in the rat, ferrous iron administered in amounts sufficient for assimilation is utilized for the synthesis of haemoglobin and for replenishing the iron reserves. The increase in haemoglobin is approximately the same whichever route of administration is employed. On the other hand, the replenishing of the iron reserves takes place more quickly if intravenous administration is used than if the iron is given orally. The formation of haemoglobin in response to iron therapy is not impaired by simultaneous administration of calcium. (Taken partly from E. Rothlin and E. Undritz: "Experimenteller Beitrag zum Eisenstoffwechsel", Third communication, Helv med Acta **13**, 460 [1946]).

Plate 9

Leucocytes: differentiation of mature elements and stem cells

Figures 43 and 44: mature leucocytes

granules of uniform size

Figure 44. Leucocytes with basophilic blue cytoplasm in normal blood films: monocytes, lymphocytes, plasma cells

normal lymphocytes.

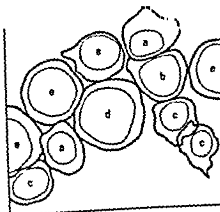
Figures 45 and 47: stem cells of leucocytes (myeloblasts) (Text on p. 42, Tables 7, 8, 9)

B. and C. above: eosinophiloblasts. B. *Large specimen with dark blue cytoplasm and a large, chromatin-covered nucleolus beneath the light patch in the nucleus. Small, round, achromatic dots can be seen in the nucleus and cytoplasm.* C. *Small, flattened specimen containing fat vacuoles, isolated achromatic dots and a large blue, glistening nucleolus. See also Figures 61, 67.*

D. top and bottom: neutrophilioblasts. Round nuclei with fairly coarse structure; *the chromatin fibres are long and the nuclei contain several blue nucleoli, surrounded by dense chromatin borders.* Left centre: *flattened neutrophilic promyelocyte II.* Right centre *segmented neutrophil.* See also Figures 71, 88, 89, 95.

G. centre: lymphoblast. *Coarse, lumpy nucleus with a distinct, blue nucleolus.* Below is a lymphocyte with cytoplasmic projection. Also present are two naked lymphocyte nuclei and a round, detached fragment of cytoplasm. In each of the crushed lymphocytes a small nucleolus has become visible. *See also Figures 115, 133.*

H above: two plasmoblasts. Dark cells with round nuclei, having dense, indistinct chromatin networks and *no visible nucleoli.* The



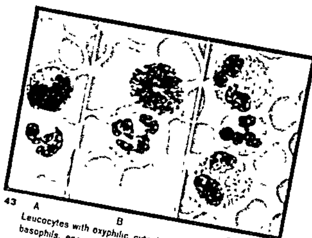
appearance, although some of them are from leukaemic patients. Apart from the confluent *nucleoli*, the basophiloblasts in A. may also be considered to be of normal appearance.

Figure 48. Differentiation by means of Unna-Pappenheim staining (Text on p. 64)

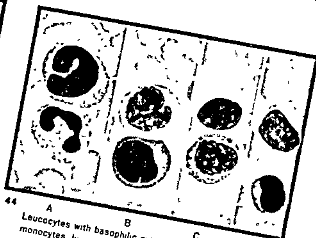
A. a) *three plasmocytes,* b) *pronormoblast,* c) *three normoblasts,* d) *eosinophilic myelocyte,* e) *immature neutrophil*

B. Plasmocyte. *The cytoplasm is filled with Russell bodies and the green nucleus is just visible on the margin at the left.*

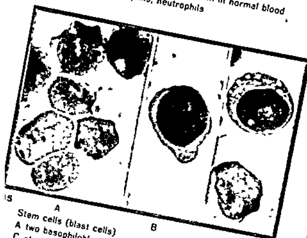
The intense red coloration is given not only by the cytoplasm of the plasma cells and by the Russell bodies but also by the cytoplasm of the normoblasts, the granules



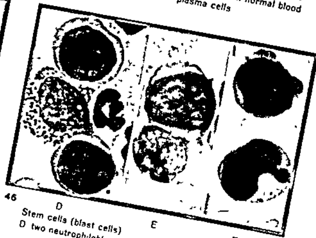
43 A B C
Leucocytes with oxyphilic cytoplasm in normal blood basophils, eosinophils, neutrophils



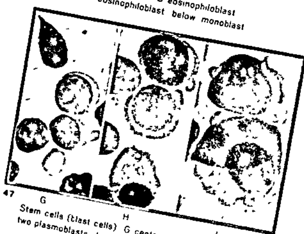
44 A B C D
Leucocytes with basophilic cytoplasm in normal blood monocytes lymphocytes plasma cells



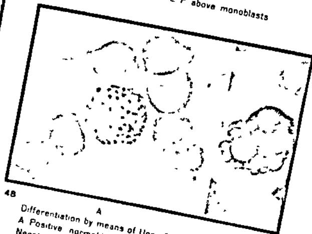
45 A B C
Stem cells (blast cells)
A two basophiloblasts B eosinophiloblast
C above eosinophiloblast below monoblast



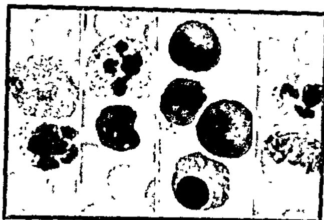
46 D E F
Stem cells (blast cells)
D two neutrophiloblasts E F above monoblasts



47 G H I
Stem cells (blast cells) G centre lymphoblast H above two plasmoblasts I above megakaryoblast



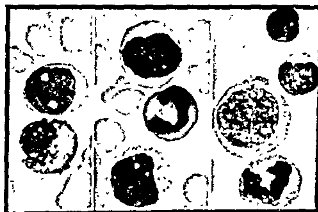
48 A B
Differentiation by means of Unna Pappenheim stain
A Positive normoblasts plasma cells
Negative neutrophils B



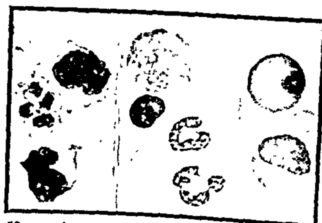
49. A B C D
Graham-Knoll peroxidase reaction,
mature leucocytes in blood and bone marrow



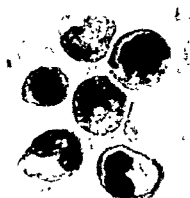
50. A
Graham-Knoll peroxidase reaction ... ...nd
monocytes and neutrophils



51. A B C
Negative Graham Knoll peroxidase reaction of stem cells (bone marrow): A neutrophiloblast, B monoblast and promonocyte, C macronormoblast



52. A B C
A Modification I of the peroxidase reaction (blood) ... II monocytes negative B and C modification II of ...peroxidase reaction only eosinophils positive



53. Modification I of the peroxidase reaction
marrow
Positive neutrophils and eosinophils



marrow

Plate 10

Leucocytes: Differentiation of leucocytes with the peroxidase reaction

Figure 49. Graham-Knoll peroxidase reaction with *mature* leucocytes (*Text on pp* 28, 29) *The peroxidase-positive* parts vary from yellowish-green to brown A. above: strongly positive segmented eosinophil A. below: segmented basophil with a few positive (greyish-brown) granules B *above*, C above, *and D.* above: five strongly positive neutrophils at *various* stages of *maturity* B. below: negative lymphocyte C *below: negative* plasma cell. D. below, *positive* monocyte
A, B and D. Blood film C. Bone marrow film

Figure 50. Graham-Knoll peroxidase reaction with mature leucocytes (Text on pp 28, 29) A. above and bottom left: two *strongly* positive segmented *neutrophils Centre: fairly strongly positive monocyte Bottom right*, weakly positive *monocyte* B. Three positive *segmented* neutrophils and a negative *monocyte.*
Healthy *individuals* Blood films.

Figure 51. Negative Graham-Knoll peroxidase reaction of stem cells (Text on pp [illegible] A. [illegible], positive *segmented* eosinophil B Above: *negative* [illegible] C [illegible] negative *macronormoblast.* The few peroxidase [illegible] from a crushed eosinophil Above, two negative [illegible]
Bone marrow films Graham-Knoll peroxidase [illegible]

As *Figures* 49, 50 *and* 51 show, the Graham-Knoll *peroxidase reaction* is usually *strongly positive in eosinophils and* neutrophils, varies from strongly positive to negative in monocytes, is usually negative in blood basophils and always *negative in the megakaryocyte-platelet system and in lymphocytes* and plasma cells. *All species* of stem *cells (blast* cells) are peroxidase *negative*

Figure 52. Special *modifications* of the *peroxidase reaction* (Text [illegible]
A. *Modification* I of the peroxidase [illegible]
a positive [illegible]
[illegible]

Using *modification* I of the *peroxidase* reaction, monocytes *can* be *identified* with *certainty*, because they give a negative *reaction* whereas the *neutrophils*, with which *they* are *most liable* to be *confused*, react *positively*
Modification II enables the eosinophils to be identified with certainty as they are the only cells which give a positive (green) reaction.

Figure 53 Modification I *of the peroxidase* reaction in *normal bone* marrow (Text on pp 28, 29). *All elements give* a strong *positive* reaction Above six *neutrophils*, the two *large* ones *on* the *right being promyelocytes* Below two *segmented eosinophils In addition*, part of a crushed *nucleus* (Gumprecht's *shadow*) may be seen at the *extreme top*
Healthy *individual* Bone marrow *film.*

Figure 54. Modification I of the *peroxidase* reaction in the *bone marrow* in *agranulocytosis* (Text on p 28) The three peroxidase-positive green cells are neutrophils, the one below on the left being a promyelocyte Neutrophils *exhibiting peroxidase-failure* were not present, so that *the three* peroxidase-negative cells *must be monocytes* Top centre: three blood platelets
Agranulocytosis with monocytosis Bone marrow film

In certain diseases, such as agranulocytosis and leukaemia, in which atypical cell forms are present, *the identification* of monocytes may be *difficult, especially in* bone marrow *films In these cases*, the appearance alone is not sufficient to enable the cells to be identified as monocytes; *they* must also give a negative result *in modification* I of the *peroxidase* reaction and the *presence of* neutrophils exhibiting peroxidase-*failure* (pp 28, 57 and Figure 94) must be excluded.

Leucocytes: Blood Basophils (basophils with soluble granulation)

Stages of development; staining characteristics; leukaemic changes; mitoses and abnormal cells

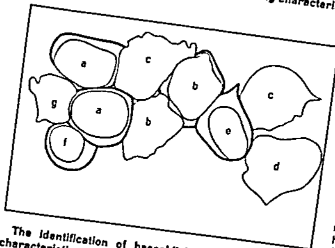


Figure 55. Basophils: stages of development (Text on p 49)

a) Basophiloblasts The nuclei have a very fine chromatin network, free from lumpiness Pale, indistinct nucleoli, with no peripheral thickening of the chromatin Narrow border of cytoplasm.
b) Basophilic promyelocytes I Similar cells, but containing a few typical basophilic-metachromatic granules
c) Mature basophils Abundant specific granulation.
d) Crushed basophiloblast.
Also present are:
e) Eosinophilic myelocyte.
f) Polychromatic normoblast.
g) Denuded, crushed nucleus of unknown origin

Basophilic leukaemia in an Arab with 59% basophils in the bone marrow and 63% basophils in the blood Bone marrow film Pappenheim staining (By courtesy of Prof. Chevallier and Dr. Marinone, Paris [7])

The identification of basophiloblasts in normal bone marrow is difficult, since their appearance is not very characteristic and the total number of basophils is small. In the rare cases of basophilic leukaemia, basophiloblasts may appear in large numbers and can then be recognized easily by comparison with the basophilic promyelocytes I. These they resemble in appearance except for the absence of the specific granulation. See also Figure 45 A.

Figure 56. Basophils: stages of development (Text on p 49) A. above and C above · basophilic promyelocytes I with dark blue cytoplasm, B Basophilic promyelocyte II, micro-form from a case of leukaemia C below and D basophilic myelocytes. The substance of the cytoplasm is pink. In A, B and C, the specific basophilic-metachromatic granules have been retained, owing to the use of Pappenheim staining In D they have been washed out during fixation prior to the peroxidase reaction The cell in D is peroxidase-negative like the majority of basophils Also to be seen are a neutrophil (A. below), several normoblasts (C. and D) and part of a peroxidase-positive neutrophil (extreme right)

A. Eosinophilia B. Chronic myelogenous leukaemia, acute phase C. Werlhof's disease D Healthy individual A C and D Bone marrow films with qualitatively normal cells, B blood film A. to C Pappenheim staining, D Graham-Knoll peroxidase reaction.

Figure 57. Segmented basophils in the blood: Pappenheim staining and toluidine blue (Text on p 50) Mature blood basophils The nuclei are segmented in contrast to the round nuclei of the mature tissue basophils, Figures 191 192. A B and C Pappenheim staining The basophilic granules have not been dissolved and cover the nuclei D Toluidine blue staining (see p 28) The granules show characteristic metachromasia, the blue colour of the stain having changed to a reddish violet. Blood film Healthy individual.

Large azurophilic granules, such as can be seen in neutrophilic and eosinophilic promyelocytes, stain less intensely with toluidine blue and show a weaker metachromasia than the true basophilic granules.

Figure 58. Segmented basophils in the blood: Giemsa staining and the peroxidase reaction (Text on p 50) Mature segmented blood basophils. A B and C Giemsa staining, D peroxidase reaction Most of the basophilic granules have been dissolved out and the cells have a diffuse reddish colour, the nuclei are clearly visible and the degree of segmentation can be easily determined In D the basophil at the top is peroxidase-negative, the one at the bottom peroxidase-positive

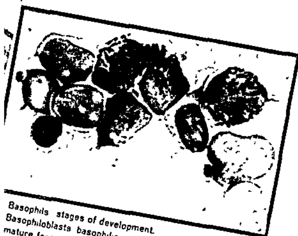
A. B and C. Healthy individual. D Chronic myelogenous leukaemia Blood films

Figure 59. Basophilic leukaemia. Mature form with segmented basophils in the blood (Text on p 50) Group of 11 segmented basophils Bottom left · twinning deformity

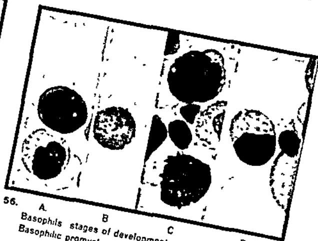
Blood film, Pappenheim staining From the same case as Figure 55 It is n … re, too … l exhibit the characteristic reddish coloration, typical of this species of blood cell.

Figure 60. Mitoses of basophils and a twinning de… … anaphases with characteristic, thick, aggregated chromosomes and obtuse … d basophil with two diploid nuclei) The basophil in C is peroxidase-negative … wing to the different staining methods used, the granules in A. and C. h… reserved In A. below, a monocyte is also visible

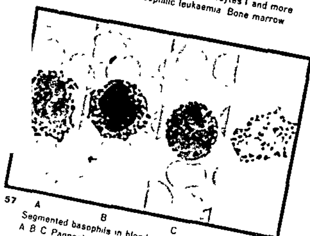
A. B and C. Healthy individuals, bone marrow films D Myeloc… …enheim staining, C. peroxidase reaction.



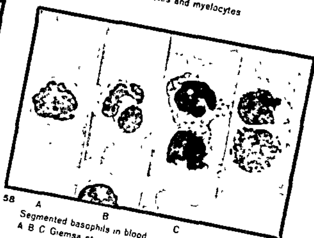
Basophils stages of development.
Basophiloblasts basophilic promyelocytes I and more mature forms in basophilic leukaemia Bone marrow



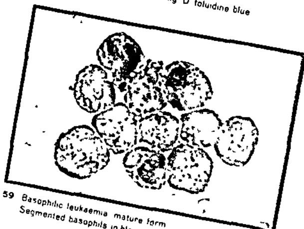
56. A B C D
Basophils stages of development
Basophilic promyelocytes and myelocytes



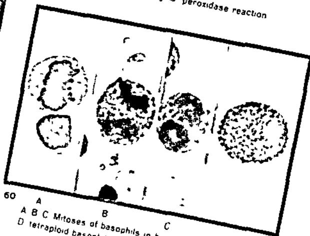
57 A B C D
Segmented basophils in blood
A B C Pappenheim staining D toluidine blue



58 A B C D
Segmented basophils in blood
A B C Giemsa staining D peroxidase reaction

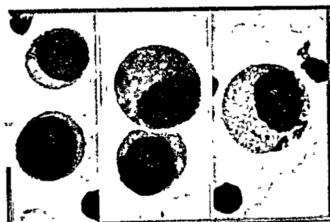


59 Basophilic leukaemia mature form
Segmented basophils in blood

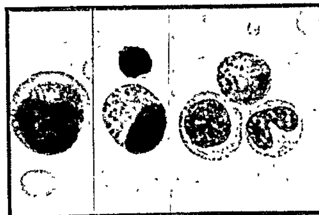


60 A B C D
A B C Mitoses of basophils in bone marrow
D tetraploid basophil with two nuclei (twinning deformity)

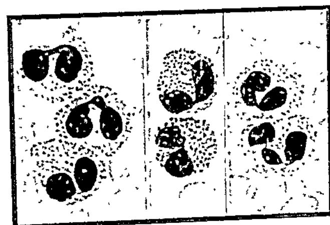
Magnification 1:1200



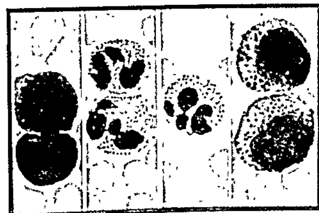
61. A B C
Eosinophils · stages of development
Eosinophiloblast, promyelocytes I and II.



62. D E F.
Eosinophils stages of development
Eosinophilic myelocytes and a metamyelocyte



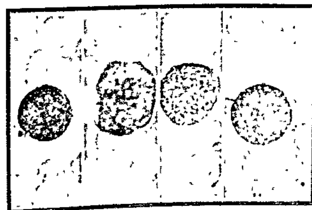
63. A B C
Segmented eosinophils in blood



64. A B C D
Eosinophils. A peroxidase reaction, B hereditary medium hypersegmentation, C hypersegmentation, pernicious anaemia, D myelocytes in blood lymphogranuloma



65. Eosinophilia in the blood in lymphogranuloma



66 A. B C D
Immature eosinophils in the blood in myelogenous leukaemia

Plate 12

Leukocytes: Eosinophils

Stages of development; anomalies; reactive and leukaemic changes

Figure 61. Eosinophils: stages of development (Text on p 50) A. below eosinophiloblast with large nucleolus, the violet colour is due to the covering of chromatin A. above and B below. eosinophilic promyelocyte I, still exhibiting achromatic dots but containing some specific granulation B above and C eosinophilic promyelocytes II with azurophilic progranulation and definitive eosinophilic granules Achromatic dots are no longer present.

Werlhof's disease with no qualitative changes in the eosinophils Bone marrow film Pappenheim staining

Figure 62. Eosinophils: stages of development (Text on p 50) D Eosinophilic promyelocyte II with eosinophilic granules localized in the centre and a few dispersed azurophilic granules E. Semi-mature eosinophilic myelocyte with eosinophilic and azurophilic granules F. bottom left mature eosinophilic myelocyte Right juvenile eosinophil Above. basophilic promyelocyte I. The cells in F. are "micro-forms" obtained from a case of chronic myelogenous leukaemia with very marked proliferation

D Lymphatic leukaemia, E. haemolytic disease of the new-born, F. chronic myelogenous leukaemia D Bone marrow film, E. and F. blood films, D and F Pappenheim staining E. Giemsa staining

Figure 63. Normal segmented eosinophils in blood (Text on p 51, Tables 6, 10, pp 41, 51) Mature eosinophils The majority contain two pouch-shaped nuclear segments, a typical feature present in 80% of all normal eosinophils A above: elongated staff-form of eosinophil C below eosinophil with three nuclear segments

Blood films with normal eosinophils A and B Pappenheim staining, C Giemsa staining

[illegible]

lapping Pernicious anaemia (Table 10) D Two "toxic" semi-mature eosinophilic myelocytes with numerous azurophilic and a few eosinophilic granules Lymphogranuloma in a six-year old child From the same case as Figures 65 and 69 C (By courtesy of the University Paediatric Clinic, Zurich, case reported by R. F Landolt [9])

Blood films A. Peroxidase reaction, B and C Giemsa staining, D Pappenheim staining

Figure 65 Eosinophilia in the blood in lymphogranuloma (Text on p 52) One staff eosinophil and eight eosinophils with medium segmentation In addition, a juvenile neutrophil can be seen below on the left

Blood film from the same case as Figures 64 D and 69 C The blood contained 122 900 leucocytes per cu mm, 90% of which were eosinophils

Figure 66 Immature eosinophils in the blood in myelogenous leukaemia (Text on p 52) Eosinophilic promyelocytes I and II As is usual, the granules of the eosinophils not only surround the nuclei but also lie above and beneath them

Acute myeloblastic phase of chronic myelogenous leukaemia From the same case as Figures 66 B, 64 A, and 89 Blood film Giemsa staining (By courtesy of the University Medical Clinic, Zurich)

Plate 13

Leucocytes: Eosinophils

(Concluded)

Leukaemic changes; mitoses and abnormal cells

Figure 67. Eosinophilic leukaemia (Text on p 52) Blood film Centre: group of five intact cells Left: eosinophiloblast Above: eosinophilic promyelocyte Below this are three eosinophilic myelocytes and, on either side, a nucleus from a crushed eosinophilic promyelocyte, the azurophilic and eosinophilic granules from which are scattered in the vicinity

Girl aged 18 The blood contained 80,000 leucocytes per cu mm., 71% of which were eosinophils This figure comprised 54% eosinophilic promyelocytes and myelocytes, and 17% segmented eosinophils. The remaining 29% was made up of 13% neutrophils and 16% lymphocytes. Pappenheim staining (By courtesy of Dr. A. Piney, London [10]).

Figure 68. Eosinophilic leukaemia (Text on p 52) Bone marrow film. Eosinophilic promyelocytes and myelocytes containing vacuoles. The vacuole in the promyelocyte at the bottom right is the largest and almost completely fills the cytoplasm Red or azurophilic granules from crushed eosinophils are scattered over the entire preparation.

From the same case as Figure 67. The bone marrow contained 97% eosinophils, 94% being promyelocytes and myelocytes, and 3% staff forms and segmented eosinophils. The remaining 3% of the cells comprised neutrophils, monocytes and lymphocytes Pappenheim staining (By courtesy of Dr. A. Piney, London [10]).

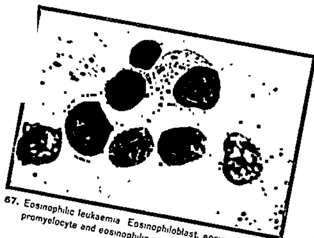
Even in bone marrow films prepared from healthy individuals the eosinophils may exhibit large vacuoles. These vacuoles contain fat released from stroma cells damaged during marrow puncture. The fat is taken up by the eosinophils and stains with Sudan III. This property is evident even in eosinophiloblasts, see Figure 45 C

Figure 69. (Text on pp 51, 52). **A. Eosinophilic myelocyte in telophase.** The chromosomes of the eosinophils are thick and aggregated Left, a staff eosinophil; above, a neutrophilic promyelocyte II; below, a lymphocyte **B. Tetraploid eosinophilic myelocyte with two diploid nuclei** (twinning deformity) **C. Tetraploid eosinophil with two segmented diploid nuclei,** the mature stage of a binuclear myelocyte like that shown in B Above: segmented eosinophil of normal size

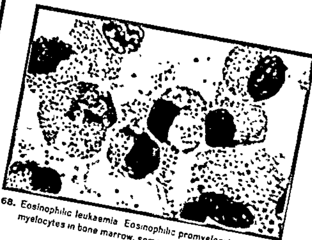
A Healthy individual, bone marrow film, Giemsa staining B Chronic myelogenous leukaemia, blood film, Pappenheim staining C. Eosinophilic reaction, blood film, Pappenheim staining

Figure 70. Highly polyploid segmented eosinophils (Text on p 52) A. Eosinophil, probably octoploid, with four segmented diploid nuclei of convolute structure Also present are some atypical neutrophiloblasts B Juvenile polynuclear eosinophil, probably 16-ploid (cf. Figure 106 B showing a neutrophil at the same stage of development with a similar degree of polyploidy) Close to the nucleus, below on the right, is a vacuole

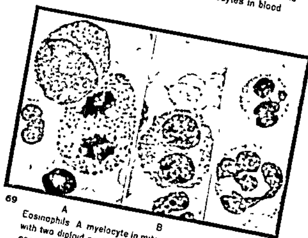
Reactive or primary myelosis in tuberculosis. From the same case as shown in Figure 106 A Bone marrow film, Pappenheim staining (By courtesy of Dr. S J Leitner, Leysin)



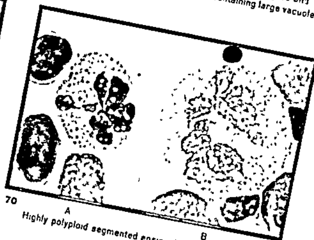
67. Eosinophilic leukaemia Eosinophiloblast, eosinophilic promyelocyte and eosinophilic myelocytes in blood



68. Eosinophilic leukaemia Eosinophilic promyelocytes and myelocytes in bone marrow, some containing large vacuoles

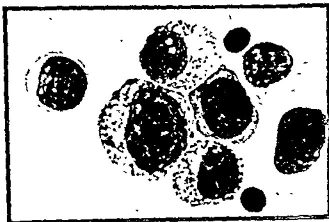


69 A B C
Eosinophils A myelocyte in mitosis B tetraploid myelocyte with two diploid nuclei, C tetraploid binuclear segmented eosinophil B and C twinning deformities



70 A B
Highly polyploid segmented eosinophils

Magnification 1:1200



71. Neutrophils · stages of development
Neutrophiloblast, promyelocytes I and II, semi-mature myelocytes and mature myelocyte.



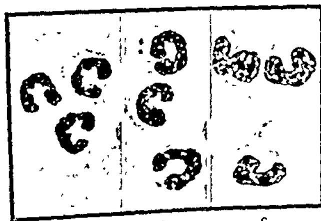
72. A B
Neutrophils · stages of development.
Myelocytes, metamyelocytes, juvenile forms, staff forms, segmented forms.



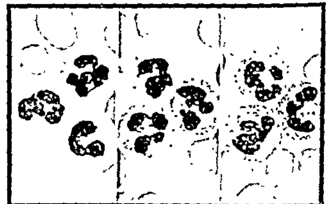
73. A B.
Neutrophils: stages of development
Juvenile forms, staff forms, segmented forms.



74. A. B
Staff neutrophils and segmented neutrophils in blood



75. A B C
Regenerative shift (shift to the left) of neutrophil
Regenerative shift (shift to the left) of neutrophil nuclei in blood A chronic pulmonary tuberculosis
B and C pneumonia, with Doehle's inclusion bodies



76 A B C
Granulation of neutrophils in blood Giemsa staining.
A no granulation B scanty and medium granulation.
C medium and excessive (toxic) granulation.

Plate 14

Leucocytes: Neutrophils

Stages of development and reactive changes

Figures 71 to 74. *Neutrophils: Stages of development* (Text on p 53)

Figure 71. [illegible] c) *neutrophil* [illegible] *myelocytes*, e [illegible] *blasts*, g) *den* [illegible] *shadow)*

Figure 72. a) Mature *neutrophilic myelocytes*, b) *neutrophilic metamyelocytes*, c) *juvenile neutrophil*, d) *staff neutrophil*, e) *segmented neutrophil*, f) *compressed basophilic normoblast*, g) part of a *neutrophilic promyelocyte* (!), h) part of an *eosinophil*

Figure 73. A [illegible] ed form. Also present are two *oxyphilic normob* [illegible]
Figur [illegible]

Figure 74. A. above: segmented neutrophil. Below, staff neutrophil with no [illegible] *neutrophils* (one on the left above, two on the right) [illegible] The segmentation [illegible] segments [illegible]
A. Healthy [illegible] Giemsa staining

Figure 75. "*Shift to the left*", or *regenerative shift*, *affecting the nuclei of the neutrophils in the* [illegible]
A. Chronic pulmonary tuberculosis (Table 11, p. 54). Three staff neutrophils [illegible] indicates the presence of chronic inflammation [illegible] chromatin [illegible] bo [illegible]

Figure 76. Granulation of the *neutrophils* in the blood: Giemsa staining (Text on pp 27, 55). A. *Neutrophils without granulation*. One is a staff form, and two are segmented (three and five segments respectively). B. Three segmented neutrophils. The uppermost one has scanty *granulation* (+), the other two medium *granulation* (++). C. One staff form (extreme right) and two segmented neutrophils. The uppermost cell has medium granulation (++), the other two profuse, so-called '*toxic granulation*' (+++).
A and B. healthy individual. C. pulmonary gangrene. Blood films

The presence of "toxic" granulation can be established with certainty only when simple Giemsa staining is used.

Plate 15

Leucocytes: Neutrophils

(Continued)

Hereditary familial anomalies and reactive changes

Figure 77. Pelger-Hušt's anomaly in man (Text on pp 54, 55, Table 12).
A. Homozygotic form. *Two mature neutrophils and two mature eosinophils, all with round nuclei composed of very coarse, lumpy chromatin*

Blood film from a two-year old girl suffering from epilepsy but otherwise normal and in good health. Both parents exhibited the heterozygotic form of Pelger-Hušt's anomaly, the only conditions under which a homozygotic individual could be produced. This is the only case so far known. Pappenheim staining (By courtesy of Dr. N. Haverkamp Begemann, Leyden [11]).
B. Heterozygotic form. *Three Pelger neutrophils: above and below, segmented form; centre, staff form The cell at the bottom has the highly characteristic pince-nez form* [12]
C. Partial carrier form. *Above: normal neutrophil with medium segmentation. Below: bisegmented Pelger neutrophil having characteristic pince-nez form. In contrast to the nuclei of normal neutrophils, the nuclei of Pelger cells are short, thick and rich in chromatin, and have a coarse, lumpy structure* [13]

B and C. Blood films from adults. Giemsa staining.

In practice, the heterozygotic manifestation is the most important, since nearly all Pelger individuals are heterozygotic.

Figure 78. Pelger-Hušt's anomaly in rabbits (Text on pp. 54, 55, Table 12)
A. Homozygotic form. [illegible]
not segmented, but withi [illegible]
B. Heterozygotic form. [illegible]
C. Partial carrier form. [illegible]
pince-nez form [16]

Blood films from rabbits exhibiting various forms of Pelger-Hušt's anomaly. Pappenheim staining (A. *By courtesy of Prof. H. Nachtsheim, Berlin*)

The neutrophils of rabbits have a marked acidophilic (pseudoeosinophilic) granulation, a secondary feature differentiating them from the neutrophils of man.

Pelger-Hušt's anomaly has identical characteristics in man and in rabbits. Secondary quantitative differences may be seen from Table 12, p. 55. The anomaly affects not only the neutrophils but all species of blood cells. However, as the changes are most noticeable in the neutrophils, this species has been chosen for the illustrations.

Figure 79. Pseudo-Pelger-cells *(Text on p 55).* **A** *above,* **B.** *und* **C.:** *two staff neutrophils and a bisegmented neutrophil (pince-nez form) These cells bear a great resemblance to true Pelger neutrophils of the heterozygotic type, but the chromatin aggregates are smaller and less sharply outlined* **A.** *below: unchanged, normal staff neutrophil. The cells contain Doehle's inclusion bodies and toxic granulation, evidence that a severe, acute infection is present.*

27 year old patient with severe enteritis. The blood contained a high percentage of Pelger-like neutrophils (48 % of all neutrophils) After recovery, only normal neutrophils were present. Pappenheim staining (By courtesy of Dr. F Heckner, Göttingen [16]).
D. *Neutrophils with round nuclei, resembling those of homozygotic Pelger Hušt's anomaly. In a rabbit with the heterozygotic form, after intravenous injection of colchicine The nuclei have a coarse structure, but are not fragmented and are more voluminous than in rabbits with the true homozygotic form of the anomaly, while the nucleus is larger in comparison with the cytoplasm, cf* Figure 78 A *Moreover, only some of the neutrophils have round nuclei The phenomenon is most pronounced about 12 hours after the administration of colchicine, and disappears again in approximately the same time. Pappenheim staining* (By courtesy of Prof H Nachtsheim, Berlin [17])

Figure 80. Hereditary hypersegmentation of neutrophil nuclei *(Text on p 56) Three neutrophils, each with four nuclear segments* (Table 11, p 54)

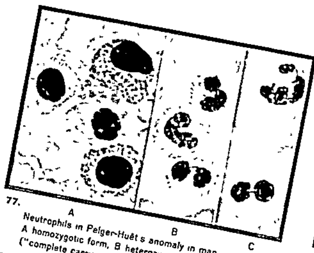
Healthy adult showing the anomaly [8]. *Blood film. Giemsa staining*

Figure 81. Hypersegmentation of neutrophil nuclei in pernicious anaemia *(Text on p 57)* **A.** *Neutrophils in pernicious anaemia of pregnancy* (Table 11, p 54) **B.** *Cryptogenic pernicious anaemia* (Table 11, p 54). *The neutrophils contain hypersegmented nuclei. In addition, in A. left, there is a target-normocyte (cf. Figure 15 A)*

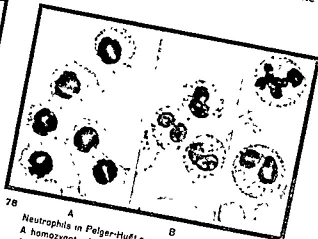
Blood films. Pappenheim staining (A. By courtesy of the University Policlinic, Basle).

Figure 82. Reactive hypersegmentation of neutrophil nuclei ("shift to the right") in sepsis (Text on p 57 Table 11 p 54).
Neutrophils with hypersegmentation of the nuclei, an extremely rare reaction in infections

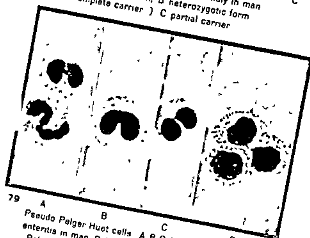
Moribund adult with phlegmon of the chest wall Blood film. Pappenheim staining (By courtesy of the University Policlinic, Basle)



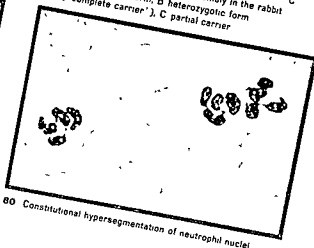
77. A B C
Neutrophils in Pelger-Huët s anomaly in man
A homozygotic form, B heterozygotic form
("complete carrier) C partial carrier



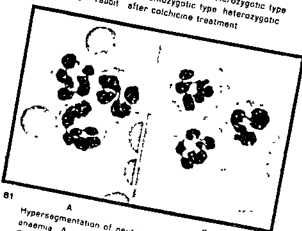
78 A B C
Neutrophils in Pelger-Huët s anomaly in the rabbit
A homozygotic form, B heterozygotic form
('complete carrier'), C partial carrier



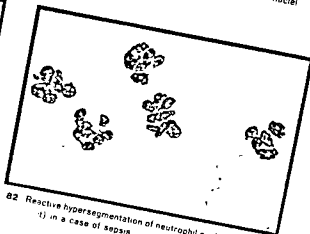
79 A B C D
Pseudo Pelger Huet cells A B C heterozygotic type
enteritis in man D homozygotic type heterozygotic
Pelger rabbit after colchicine treatment



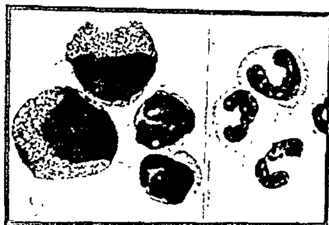
80 Constitutional hypersegmentation of neutrophil nuclei



81 A B
Hypersegmentation of neutrophil nuclei in pernicious
anaemia A pernicious anaemia of pregnancy
B cryptogenic pernicious anaemia



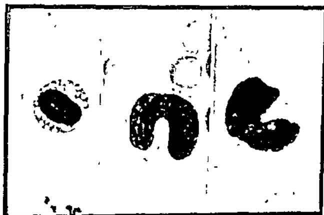
82 Reactive hypersegmentation of neutrophil nuclei (shift to the
:t) in a case of sepsis



83. A B.
Neutrophils and monocytes:
A. In the bone marrow in agranulocytosis.
B In the blood in pneumonia.



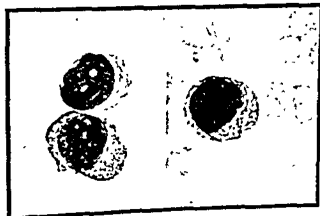
84. Immature neutrophils with excessive granulation in the bone marrow in agranulocytosis ("toxic marrow")



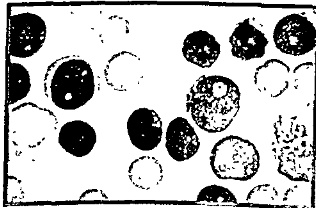
85. A B C.
Pathological neutrophils in the blood
A vacuolated neutrophil, B giant metamyelocyte in myelogenous leukaemia, and C. in pernicious anaemia.



86 A B C
Giant metamyelocytes in the bone marrow in pernicious anaemia· A neutrophilic metamyelocytes B eosinophilic metamyelocyte, C basophilic metamyelocyte



87. A. B
Neutrophilic promyelocytes II with Auer s rods in leukaemia



88. Acute myelogenous leukaemia with small neutrophiloblasts (micromyeloblasts) Blood

Plate 16

Leucocytes: Neutrophils

(Continued)

Comparison with monocytes; reactive changes; pathological forms; leukaemias

Figure 83. Neutrophils and monocytes (Text on p 57) A. left: two neutrophilic myelocytes with profuse toxic granulation Right two considerably smaller monocytes; being from a thicker portion of the film they were unable to flatten out in their usual manner. B Two staff neutrophils (below) and a monocyte with a similarly shaped nucleus The monocyte may be distinguished from the neutrophils by its blue cytoplasm, which shows up particularly well with Giemsa staining and by the looser structure of the nucleus.

A. Agranulocytosis, bone marrow film, Pappenheim staining B Pneumonia, blood film, Giemsa staining

Monocytes of the bone marrow may easily be confused with neutrophilic promyelocytes and myelocytes. In the blood, monocytes with deep nuclear indentations may be mistaken for juvenile or staff neutrophils, cf Figure 44 A.

Figure 84. Immature neutrophils with profuse granulation in the bone marrow in agranulocytosis ("toxic marrow" pp 16, 27, 56) Neutrophils with profuse, toxic granulation (+++) Two semi-mature myelocytes, two mature myelocytes (almost metamyelocytes), four juvenile myelocytes and three staff forms

Agranulocytosis in the recovery phase Bone marrow film Weak Pappenheim staining

Figure 85. Pathological neutrophils in the blood (Text on pp 16, 56) A. Vacuolated neutrophil with staff nucleus, the two ends of which have become accidentally superimposed. B and C. Giant neutrophilic metamyelocytes, the one in C. containing an indistinct vacuole in the upper part of the cytoplasm.

A. and B Chronic myelogenous leukaemia, Pappenheim staining C. Pernicious anaemia, Giemsa staining Blood film.

Figure 86 Giant metamyelocytes in the bone marrow in pernicious anaemia (Text on p 16) A. Two giant neutrophilic metamyelocytes B A giant eosinophilic metamyelocyte, for comparison C A giant basophilic metamyelocyte for. comparison Also present are a normal basophil and a segmented neutrophil The nuclei of the basophils show the characteristic reddish tinge

Untreated pernicious anaemia Bone marrow film Giemsa staining

Giant metamyelocytes are very often observed in pernicious anaemia, but they may also occur in other diseases, especially in other types of anaemia and in myelosis (Figure 85 B.) **They are the precursors of the corresponding hypersegmented cells.**

Figure 87. Neutrophilic promyelocytes II with Auer rods in leukaemia (Text on p 55) A. Two neutrophilic promyelocytes, the lower cell containing normal azurophilic progranulation, the upper one azurophilic needles, the so-called "Auer rods". B Neutrophilic promyelocyte II with Auer rods In addition, normocytes with central depressions, so-called anulocytes (cf Figures 15 B. and 88), a symptom of the concomitant hypochromic anaemia

Acute myelogenous leukaemia with predominance of neutrophilic promyelocytes II. Pappenheim staining B. From the same case as Figures 91 and 92. (A. By courtesy of the University Medical Clinic, Basle B By courtesy of the University Medical Clinic, Zurich).

Auer rods are found only in neutrophilic promyelocytes and only in leukaemic patients.

Figure 88. Acute myelogenous leukaemia with production of small neutrophiloblasts (micromyeloblasts) (Text on p 56) Neutrophiloblasts, the majority of which are small (approximately the size of an erythrocyte) and consist of almost naked nuclei This is due to the large number of cells and the acute character of the disease An abnormal feature is the presence of only one or two nucleoli (pathological reduction in the number of the nucleoli, see pp 43, 63), in the majority of cells no nucleoli at all are visible To the right of the centre is a neutrophilic promyelocyte I Characteristic azurophilic progranulation is present in the nuclear indentation and the nucleus contains a large nucleolus, probably produced by the fusion of several nucleoli of normal size The presence of typical neutrophilic promyelocytes II, i e. of other more nearly mature neutrophils, excluded a diagnosis of lymphatic leukaemia Such cases can also be distinguished by the fact that the granulated stages give a positive peroxidase reaction whereas the lymphocytes are negative In the top right hand corner is a cell in mitosis and below, a Gumprecht's shadow from a neutrophiloblast. Anulocytes are also present.

Blood film Pappenheim staining

Plate 17

Leucocytes: Neutrophils

(Continued)

Leukaemias

Figure 89. Myeloblastic crisis in chronic myelogenous leukaemia. A. above four neutrophiloblasts. The nuclei have a loose chromatin network and contain two or three nucleoli most of which are rather indistinct. Below: mature neutrophilic myelocyte Pappenheim staining. B Graham-Knoll peroxidase reaction. Like all blast cells, the neutrophiloblasts are peroxidase negative In the centre are three somewhat more mature peroxidase-positive neutrophils.

Myelosis with a blood leucocyte count of 103,600 per cu mm, 64% being neutrophiloblasts. Blood films. From the same case as *Figures 56 B, 64 A and 66 (By courtesy of the University Medical Clinic, Zurich)*

Figure 90. Acute myelogenous leukaemia with predominance of neutrophilic promyelocytes I containing Auer rods. (Text on p. 56) A Three well spread out neutrophilic promyelocytes I, each containing one Auer rod in the nuclear indentation near the lower nuclear pole. The nuclei show atypical indentations ("paraforms") and contain large nucleoli formed by coalescence (pathological reduction in the number of nucleoli) Giemsa staining B Graham-Knoll peroxidase reaction Six similar cells to those in A, but poorly spread out. In the nuclear indentations, they are peroxidase positive These promyelocytes I differ from their precursors, the neutrophiloblasts, only in the presence of a single Auer rod and in giving a positive peroxidase reaction

Leukaemia with 5% neutrophiloblasts and 58% promyelocytes I, the absolute leucocyte count being normal Blood film.

A. and B. are from the same blood film, the slide having been cut lengthwise. One half was stained with Giemsa stain and the other was subjected to the peroxidase reaction. The cells in B., being in a thicker part of the film, are smaller than those in A. and show fewer details. This figure illustrates the dependence of the cellular diameter on the thickness of the film

Figure 91. Acute myelogenous leukaemia with predominance of neutrophilic promyelocytes II (Text on p 56) A. Nest of neutrophilic promyelocytes II. The abundant azurophilic progranulation is characteristic The nucleoli are barely visible Pappenheim staining B Graham-Knoll peroxidase reaction. The cells are strongly positive

Leukaemia with a blood leucocyte count of 4,500 per cu mm, 84% being neutrophilic promyelocytes II Bone marrow films. From the same case as Figures 87 B and 92.

Figure 92. Auer rods in neutrophilic promyelocytes II (Text on p. 56). Some of the promyelocytes II contain only azurophilic granules. Auer rods are present in three cells on the left. The crushed cell at the top contains a few azurophilic granules as well as Auer rods.

From the same case and preparation as Figure 91 A Bone marrow film Pappenheim staining

Figure 93. Acute myelogenous leukaemia with atypical monocyte-like neutrophilic promyelocytes II (Text on p 57) A Promyelocytes II with lobulate nuclei like those of the monocytes Giemsa staining The azurophilic progranulation does not show up as well as in Figure 91 A., where Pappenheim staining was used B. Graham-Knoll peroxidase reaction The cells are strongly *positive and must therefore be identified as promyelocytes II and not as monocytes.*

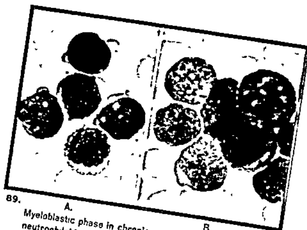
Leukaemia with a blood leucocyte count of 4,900 per cu mm, 86% being promyelocytes Blood films. (By courtesy of the University Medical Clinic, Zurich)

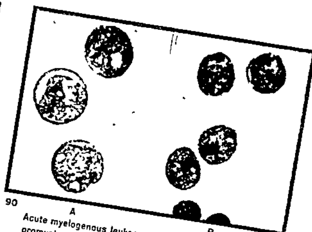
Leukaemia with atypical neutrophilic promyelocytes II ("paraforms") differs from monocytic leukaemia in that some or all of the pathological cells give a positive peroxidase reaction. If only some of the neutrophils are positive, as in Figure 94, the phenomenon is termed "peroxidase failure".

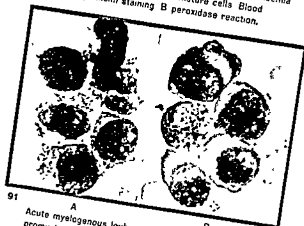
Figure 94. Acute myelogenous leukaemia with atypical, monocyte-like neutrophilic promyelocytes II, some of which are peroxidase negative. (Text on pp 28, 57, 60) A Group of six monocyte-like neutrophilic promyelocytes The cells are all similar in appearance but three give a strongly positive peroxidase reaction, while the other three are negative Below: polychromatic normoblast. B Two segmented neutrophils The lower one gives a strongly positive peroxidase reaction, the upper one is negative

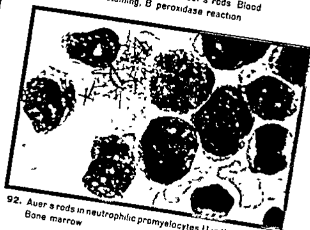
Acute myelogenous leukaemia (with atypical promyelocytes II) Blood film, Graham-Knoll peroxidase reaction (By courtesy of the University Policlinic, Basle)

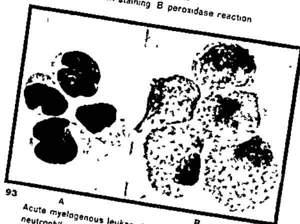
A partial peroxidase-failure in the neutrophils can only be established with certainty when, as in this case, some of the mature segmented neutrophils in the blood also show a negative reaction (In bone marrow films the neutrophils may become negative as a result of maceration but this is without significance)

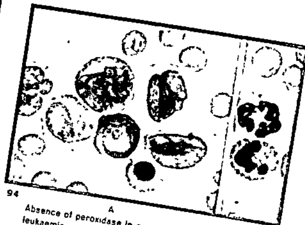

89. A. B
Myeloblastic phase in chronic myelogenous leukaemia neutrophiloblasts and more mature cells Blood
A. Pappenheim staining B peroxidase reaction.


90 A B
Acute myelogenous leukaemia with neutrophilic promyelocytes I containing Auer's rods Blood
A Giemsa staining, B peroxidase reaction


91 A B
Acute myelogenous leukaemia with neutrophilic promyelocytes II Bone marrow
A Pappenheim staining B peroxidase reaction


92. Auer s rods in neutrophilic promyelocytes II in the same case Bone marrow


93 A B
Acute myelogenous leukaemia with atypical monocyte-like neutrophilic promyelocytes II Blood
A Giemsa staining B peroxidase reaction


94 A B
Absence of peroxidase in certain neutrophils in a case of leukaemia with atypical neutrophilic promyelocytes

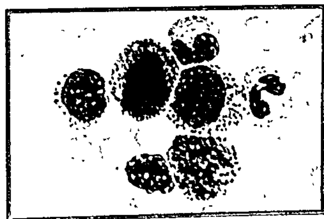
Magnification 1:1200



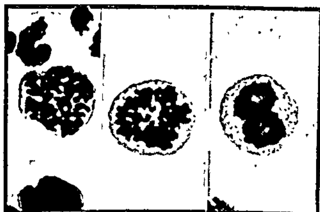
95. Chronic myelogenous leukaemia: various stages of development of the neutrophils in the blood.



96. Myelogenous leukaemia with extremely chronic course; some of the neutrophils are hypersegmented. Blood



97. Chronic myelogenous leukaemia in Pelger-Huët's anomaly. Blood



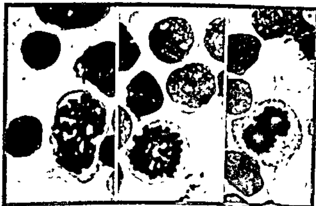
98. A. B C

Mitoses of normal neutrophils in bone marrow. A neutrophiloblast in prophase. B promyelocyte I in metaphase, C promyelocyte I in anaphase



99. D E. F

D promyelocyte II in metaphase, E. in telophase, F. myelocyte in metaphase



100 A. B C

Mitoses of neutrophiloblasts in neutrophiloblastic leukaemia: A in prophase, B in metaphase, C. in anaphase. Bone marrow

Plate 18

Leucocytes: Neutrophils
(Continued)

Leukaemias; mitoses

Figure 95. Chronic myelogenous leukaemia (Text on pp 15, 56). Neutrophilic leucocytes *in various stages of development*: metamyelocytes, juvenile neutrophils, staff forms, segmented neutrophils and top right, a *neutrophiloblast*. Below, part of a segmented *eosinophil*; extreme left, a normoblast.
Leukaemia with a leucocyte count of 350,000 per cu mm Blood film Pappenheim staining. From the same case as Figures 62 F. and 69 B (By courtesy of the University Policlinic, Basle)

The simultaneous overproduction of several species of blood cells is characteristic of chronic myelogenous leukaemia. See also Figure 189

Figure 96 Chronic myelogenous leukaemia of very long standing (Text on pp 15 56) One juvenile neutrophil and five neutrophils with medium segmentation and hypersegmentation of the nuclei.
Myelosis of more than 10 years' duration, with a leucocyte count of approximately 50,000 per cu.mm. at the time of the investigation Blood film Giemsa staining

Figure 97. Chronic myelogenous leukaemia in Pelger-Huët's anomaly (Text on p 55) One neutrophilic promyelocyte, two semi-mature and two mature myelocytes, one juvenile neutrophil and one staff neutrophil All stages show the nuclear structure characteristic of Pelger-Huët's anomaly
Heterozygotic form of Pelger-Huët's anomaly ("complete carrier") combined with chronic myelogenous leukaemia (leucocyte count approximately 100 000 per cu mm). Blood film (By courtesy of the University Medical Clinic, Zurich, case reported by W Huber [18]).
These must be true Pelger cells and not pseudo-Pelger cells because the nuclei exhibit the typical structure, even in the early stages of development, and because the family history was positive, the daughter of the patient also showing the anomaly

Figures 98 and 99. Mitoses of normal neutrophils in the bone marrow (Text on p. 53). A. Prophase, B. D F. metaphases, C anaphase, E. telophase. A. *Neutrophiloblast* with ungranulated cytoplasm. B. and C. Promyelocytes I. These cells are no larger than neutrophiloblasts, and the cytoplasm is also blue, but contains a few azurophilic granules D. and E. Promyelocytes II These are larger than promyelocytes I and contain more abundant granulation. F Mature myelocyte. Pink cytoplasm like that of mature neutrophils.
A.B C.D. and F. Werlhof's disease, E. apoplexy Normal neutrophils Bone marrow films. Pappenheim staining

Figures 98 and 99 demonstrate a rule of general validity for the mitosis of blood cells (cf. Figures 32 A , 32 B., 37, 38 and 124) **in young cells and in the early phases of division, the chromosomes are well separated and therefore easier to distinguish than in more mature cells and in the later phases of mitosis, when they lie closer together and are often aggregated.**

Figure 100. Mitosis in neutrophiloblastic leukaemia (Text on p 56) A. Prophase, B metaphase, C. anaphase A. left, two oxyphilic normoblasts with well-defined nuclear structure; B two neutrophiloblasts, the upper one being particularly easy to distinguish by its blue, sharply defined nucleoli; C. above and below, Gumprecht's shadows of smudged neutrophiloblasts The neutrophiloblasts in interphase are smaller than normal and their mitoses are therefore also smaller than the normal mitoses depicted in Figure 98 The chromosomes lie closer together The reduction in size of the cells is possibly due to the marked increase in numbers and to their more rapid production
Acute myelogenous (microneutrophiloblastic) leukaemia with a blood count of 62,400 leucocytes per cu mm Film prepared from bone marrow very rich in cells Pappenheim staining (By courtesy of Prof Gigon Basle).

Plate 19

Leucocytes: Neutrophils

(Concluded)

Mitoses and deformed cells

Figure 101. Prophases of special neutrophilic promyelocytes. *A. Pelger-promyelocyte with fewer and thicker chromosomes than normal promyelocytes In addition, segmented eosinophil and a normoblast with almost completely denuded nucleus B. Giant myelocyte in pernicious anaemia.*

A Heterozygotic form of Pelger-Huët's anomaly, complete carrier. Bone marrow film. Pappenheim staining B. Pernicious anaemia before treatment. Bone marrow film Giemsa staining The azurophilic granulation is more clearly visible in A. than in B, owing to the use of Pappenheim staining

Figures 102 and 103. Tetraploid neutrophils with two diploid nuclei, so-called twinning deformities (Text on p 56) *A. Binuclear myelocyte, transition stage to metamyelocyte, B and C binuclear juveniles, D. binuclear segmented neutrophil with symmetrically situated nuclei E. F. and G above. binuclear segmented neutrophils with entangled, asymmetrically situated nuclei, below: normal segmented neutrophils. In addition, in A. normocyte with central acidophilic stippling, see Figure 16*

A. to D. chronic myelogenous leukaemia. E. to G healthy individuals. Blood films. D Pappenheim staining, other films Giemsa staining

Twinning deformities of segmented neutrophils owe their apparent hypersegmentation to the fact that they contain two nuclei; they can be distinguished from simple diploid cells exhibiting true hypersegmentation by the fact that they are twice as large (cf. Figures 80 to 82).

Figure 104. Atypical mitoses of neutrophilic promyelocytes (Text on p. 56) *A Neutrophilic promyelocytes in prophase with atypical irregular disposition of the chromosomes Owing to the use of Giemsa staining, the granules are only just visible B Binuclear (tetraploid) neutrophilic promyelocyte I in mitosis (double metaphase) This cannot be the anaphase of a normal cell because the spaces in the chromosome asters are too large The symmetrical arrangement of the granules on either side is striking A mitosis of this type gives rise to a cell with four nuclei C. Large promyelocyte I exhibiting an unknown degree of polyploidy since only a giant aggregate of tangled chromosomes can be distinguished The lower pole of this cell is occupied by a rosette of vacuoles, cf. Figures 106 A. and 165 K.; a protein-like substance is probably present.*

A. Chronic myelogenous leukaemia. Bone marrow film, Giemsa staining (By courtesy of the University Medical Clinic, Zurich). B Werlhof's disease in a child. Bone marrow film. Pappenheim staining (By courtesy of the University Children's Hospital, Basle) C. Neutrophilic promyelocytic leukaemia Blood film Pappenheim staining (By courtesy of the University Policlinic, Basle).

Figure 105. Polyploid neutrophilic promyelocytes I (Text on p 56) *A Tetraploid promyelocyte I with two diploid nuclei. B right: octoploid promyelocyte I with one tetraploid and two diploid nuclei. This has arisen from a precursor containing two diploid nuclei, one of which underwent normal mitosis and divided completely while the other underwent endomitosis, remaining undivided and therefore as large as the two other nuclei together (cf. Figure 159 right) Further nuclear division and maturation of such atypical cells without division of the cytoplasm results in monsters such as that in Figure 106 B., taken from the same case On the*

[illegible]

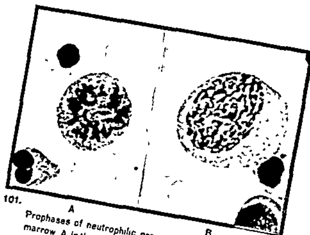
Medical Clinic, Basle)

Figure 106 Highly polyploid neutrophils (probably 16-ploid) (Text on p 56). *A. Promyelocyte II with eight, probably diploid nuclei It contains a number of large vacuoles, including a rosette-shaped group below on the right. In the centre, beginning oxyphilia B Neutrophilic metamyelocyte or juvenile, containing two probably octoploid nuclei The cell in A resembles a promegakaryocyte with eight nuclei, but contains azurophilic granulation in cytoplasm which is still basophilic; the granulation of the promegakarycytes does not appear until the cytoplasm is polychromatic. Besides, the same preparation also contained other neutrophils which were of similar appearance but were less highly polyploid or normal diploid promyelocytes In addition, it contained some unmistakable eosinophils exhibiting the same high degree of polyploidy* (see Figure 70 B). *The cell in Figure 106 B also provides proof of the existence of such highly polyploid neutrophils, for it is impossible to confuse it with any other cell Finally, Figure 106 A. cannot be an osteoclast, since,*

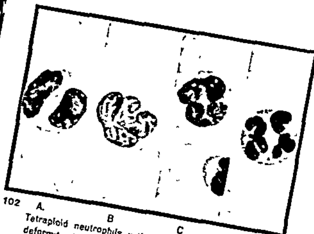
[illegible]

B. From the same case and preparation as [illegible]

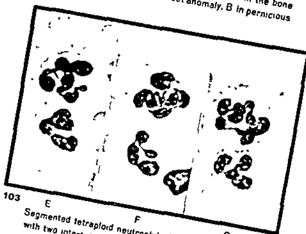
Giant cells, such as those in Figures 105 B and 106, are unable to reach the peripheral blood, since they are stopped, at the latest, by the relatively narrow capillaries of the lungs.



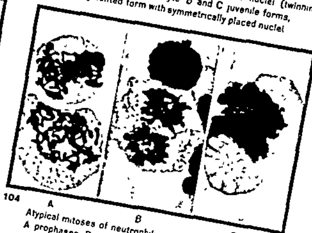
101. A B
Prophases of neutrophilic promyelocytes in the bone marrow A. in the Pelger-Huët anomaly, B in pernicious anaemia



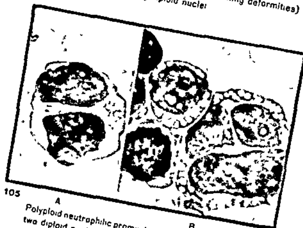
102 A. B C D
Tetraploid neutrophils with two diploid nuclei (twinning deformities) A. myelocyte B and C juvenile forms, D segmented form with symmetrically placed nuclei



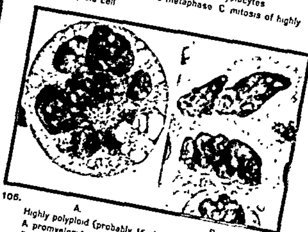
103 E F G
Segmented tetraploid neutrophils (twinning deformities) with two intertwining diploid nuclei



104 A B C
Atypical mitoses of neutrophilic promyelocytes A prophases B double metaphase C mitosis of highly polyploid cell

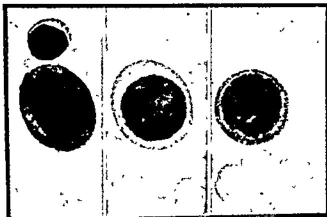


105 A B
Polyploid neutrophilic promyelocytes) A tetraploid twin with two diploid nuclei B octoploid triplet with one tetraploid and two diploid nuclei

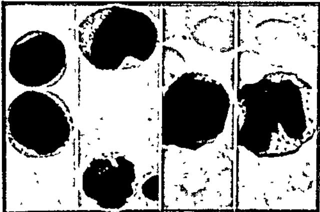


106. A. B
Highly polyploid (probably 16-ploid) neutrophils
A promyelocyte with eight diploid nuclei
B juvenile neutrophil with two octoploid nuclei

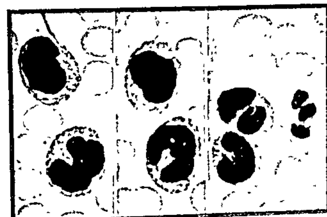
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107. A. B C.
Monocytes: stages of development
A and B. monoblasts, C promonocyte.



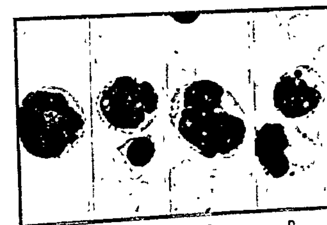
108. D E F. G
Monocytes: stages of development
Monoblast, promonocytes.



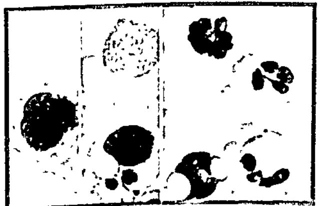
109. A. B C
Mature monocytes with slightly indented nuclei Blood.



110. D E F
Mature monocytes. D and E with deeply indented nuclei, F with segmented nucleus Blood



111. A. B C. D
Promonocytes and monocytes containing remnants of ingested material, in blood in haemolytic disease of the newborn. Giemsa staining



112. E. F G
E and F the same peroxidase reaction G monocytes and neutrophils showing phagocytosis of erythrocytes in vitro

Plate 20

Leucocytes: Monocytes

Stages of development and phagocytic forms in the blood

Figure 107. Monocytes: stages of development (Text on pp 57, 58) A. and B Monoblasts. A. Younger form · intensely basophilic cytoplasm, distinct nucleoli B More mature form: paler cytoplasm, indistinct nucleoli C Promonocyte with irregularly shaped nucleus Owing to the staining method used, the granules are only faintly visible Negative peroxidase reaction See also Figure 51 B In A. there is also a lymphocyte containing a nucleolus.

Marked monocytic reaction in agranulocytosis Bone marrow film Qualitatively normal monocytes Graham-Knoll peroxidase reaction From the same case as Figures 54, 124, 133 A., 148 B and 229 A.

Figure 108. Monocytes: stages of development (Text on pp 57, 58) D above. typical monoblast with indistinct nucleoli, somewhat irregularly shaped nucleus and ungranulated basophilic cytoplasm Below promonocyte E. above, F. and G Promonocytes in various stages of maturity In E., below, there is also a vacuolated atypical mature monocyte In D. and E., the relatively coarse azurophilic granules of the promonocytes are only feebly visible, owing to the use of Giemsa staining, whereas in F. and G they show up very plainly since Pappenheim staining was employed

D and E. Haemolytic disease of the new-born From the same case as Figures 62 E., 111, 112 E. and 112 F Blood film Giemsa staining F. and G Eosinophilia Bone marrow film Pappenheim staining The promonocytes reproduced are normal in appearance

Figures 109 and 110. Mature monocytes in the blood (Text on p 58) A. to E. Mature normal monocytes with indented nuclei of various widths F Mature monocyte with segmented nucleus The definitive, azurophilic granulation has a fine, cloud-like appearance, and is shown up more clearly by the Pappenheim staining used in A. and B than by the Giemsa staining in C. to F The cytoplasm itself has a pale pigeon-blue colour. Also to be seen are a segmented neutrophil in C on the right, a lymphatic plasma cell in F. above, and a staff neutrophil in F. below In C, the diameter of the monocytes is no greater than that of the neutrophil, because the cells are lying in a thick portion of the film, as is evident from the superimposition of the erythrocytes In thin portions of films, the monocytes have a greater diameter than the neutrophils only because they flatten out to a greater degree

A. to E. Healthy adults F Lymphatic glandular fever in a child A. and B Pappenheim staining C. to F. Giemsa staining

Figures 111 and 112. Promonocytes and monocytes containing remnants of ingested material in blood (Text on p 58) A. to C promonocytes and D two monocytes containing remnants of ingested material The round, dark violet, homogeneous inclusion bodies, some of which are lying above the nuclei, are nuclear remnants from ingested cells Furthermore, the monocyte in D below has taken up two normocytes (erythrocyte phagocytosis) the lower of which has lost most of its colour. E. Promonocyte containing the remains of an ingested neutrophil, the liquefied and condensed nucleus of which is surrounded by peroxidase-positive cytoplasm The remainder of the cytoplasm of the monocyte is still peroxidase negative Monocytes give a positive peroxidase reaction throughout the cytoplasm only after more complete digestion of peroxidase-positive cells F. below mature monocyte containing a vacuole and two ingested normoblasts, the cytoplasm of the latter has been partly extruded This monocyte is peroxidase negative because it has taken up only negatively reacting cells, its relatively large size and comparatively small, rounded nucleus are characteristic features of ageing atrophic monocytes which have performed a great deal of phagocytosis or athrocytosis In F above there is also a peroxidase-positive neutrophil The erythrocytes in E. and F show a dirty greyish-red coloration due to the peroxidase reaction G bottom left monocyte containing two ingested normocytes Above normal monocyte On the right, two neutrophils containing ingested normocytes (erythrocyte phagocytosis)

A to F Haemolytic disease of the new-born Blood films A to D Giemsa staining E and F Graham-Knoll peroxidase reaction G Blood film of a mixture containing erythrocytes and leucocytes of blood group AB and blood plasma of group O, from healthy persons Pappenheim staining (By courtesy of Dr W Baumgartner, Interlaken [19])

Figures 108 D, 108 E., 111, 112 E. and 112 F demonstrate that in haemolytic disease of the new-born not only juvenile erythrocytes (see Figure 22), but also monoblasts and promonocytes gain entrance to the peripheral blood The presence in the blood stream of juvenile forms of other leucocytes can also be observed The experiment in Figure 112 G shows that when blood from different blood groups is mixed, phagocytosis of erythrocytes may be observed not only in monocytes but also in neutrophils

Plate 21

Leucocytes: Monocytes

(Continued)

Phagocytosis and athrocytosis (storage) in the blood and bone marrow

Figure 113. Monocytes containing remnants of ingested material in blood in endocarditis lenta (Text on p 58) Group of very thinly spread out monocytes Cell on left and upper cell in the centre: normal specimens To the right: highly vacuolated monocyte. Lower cell in the centre and cell at bottom of illustration: monocytes with peroxidase-positive remnants of cytoplasm from ingested cells. On the extreme right, part of a peroxidase-positive neutrophil. The erythrocytes are stained greyish-red from the peroxidase reaction

Endocarditis lenta. Film from ear-lobe blood. Graham-Knoll peroxidase reaction. From the end portion of the film (By courtesy of Prof Gigon, Basle)

Phagocytic monocytes are very liable to rupture during the preparation of blood films, especially at the thin end of the film It is to be noted that only the ingested material gives a positive peroxidase reaction (cf. Figure 112 E) The finding of such cells in the blood of adults indicates the presence of a septic disease, and is especially pathognomic of endocarditis lenta. These forms are best seen in blood taken from the previously undisturbed ear-lobe, where the monocytes accumulate, constituting about 40% of the leucocytes even in healthy subjects. Phagocytic monocytes are most likely to be found in films prepared from the first drop of blood and towards the edges and ends of the films.

Figure 114. Monocytes containing remnants of ingested material and large numbers of vacuoles, in blood in meningococcal sepsis (Text on p 58). A. and B Monocytes with small vacuoles Some of the vacuoles contain minute violet inclusion bodies, probably remains of ingested meningococci The lower monocyte in A. also contains the necrobiotic (homogeneous, liquefied) remains of the nucleus of an ingested cell In B above and to the right, twinning deformity with overlapping nuclei.

Meningococcal sepsis (Waterhouse-Friderichsen's syndrome) in a child. Blood film with 85% monocytes, 60% containing small vacuoles Pappenheim staining. (By courtesy of the University Children's Hospital, Basle)

Figure 115. Phagocytic and athrocytic (storage) monocytes in the bone marrow in malaria (Text on p 59) A. and B Numerous typical monocytes containing stored dark brown pigment from malarial parasites The monocyte above and to the right in B. also contains remnants of the nucleus of an ingested cell, while the monocyte below contains a normocyte C. Monocyte; the cytoplasm gives a positive (blue) Turnbull blue reaction, showing the presence of stored iron, and contains a few pigment granules

A. and B Estivo-autumnal malaria Bone marrow film with 40% monocytes Pappenheim staining (By courtesy of Dr. Perret-Gentil, Basle). C Tertian malaria Bone marrow film. Turnbull blue reaction

Figure 116. Monocytes containing ingested and stored material, from embryo blood and from adult bone marrow (Text on p. 59) A. A so-called "embryo macrophage" (older phagocytic monocyte) containing two promonocytes The cytoplasm contains numerous vacuoles, especially in the vicinity of the ingested cells. These both have completely intact borders and nuclei. All three cells are monocytes, since they all have blue cytoplasm and typically shaped nuclei and all give negative peroxidase reactions The monocyte is unable to digest its own stem cells, one juvenile monocyte having even undergone division after being ingested Of interest are the marked increase in size of the phagocytic monocyte and its tenuous atrophic nucleus B Monocyte containing vacuoles and remnants of ingested cells, a so-called athrophagocyte As in A., the nucleus has the characteristic thin, flattened appearance and is poor in chromatin. Also visible are five oxyphilic normoblasts, some with structureless nuclei, and one juvenile neutrophil

A Three month old embryo. Film prepared from umbilical blood Graham-Knoll peroxidase reaction. B Healthy adult. Bone marrow film Pappenheim staining

The vacuoles of athrocytic monocytes usually contain a protein-like substance, which may be colourless or coloured depending upon the concentration and material. In the human embryo, in primitive vertebrates and in evertebrates, these large monocytes, containing ingested and stored material, are found circulating in the blood stream. In the human adult, however, although they are always present in the haemopoietic organs, they find their way into the blood stream only under pathological conditions.

Figure 117. Monocytes containing ingested material, from the bone marrow (Text on p 59) A. Monocyte containing remnants of the nuclei of ingested cells. B binuclear monocyte containing numerous ingested sickle cells (drepanocytes) — a typical finding in the bone marr[illegible] A is a younger cell with a nucleus of loose structure. B is an old cell the nuclei being rounded and atrophic. [illegible] cell anaemia

A Werlhof's [illegible]

Bone marrow film [illegible]

Figure 118. Varying degrees of athrocytosis (storage) (Text on p 59) A. Promonocyte with azurophilic progranulation Typical indented nucleus, early phagocytosis Slight increase in cell volume B Senile monocyte containing an abundance of stored material and nuclear remnants from ingested cells Final stage of a storage cell. The large size of the cell and the small rounded nucleus are characteristic

Plasmocytoma. Bone marrow film with no qualitative changes in the monocytes Pappenheim staining

Stored substances usually contain protein and may be colourless as in Figure 118. However, they can also be acidophilic or basophilic and then stain pink or blue. Moeschlin [20] has referred to monocytes containing stored material which has stained blue ("blue pigment macrophages").

Magnification 1 1200



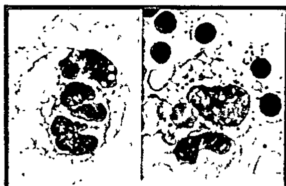
113 Monocytes containing remnants of ingested material, in blood in endocarditis lenta, peroxidase reaction



114. A B
Monocytes containing remnants of ingested material and numerous vacuoles, in blood in meningococcal sepsis



115. A B C
A. and B Monocytes containing pigment from Plasmodium falciparum, Pappenheim staining C Sideromonocyte in tertian malaria, Turnbull blue reaction Bone marrow



116. A B
Phagocytosis and athrocytosis Monocytes containing ingested and stored material A in the blood of an embryo, B in the bone marrow of an adult.



117. A B
Monocytes containig ingested material in bone marrow
A. slight increase in cell volume B phagocytosis of sickle cells with marked increase in cell volume



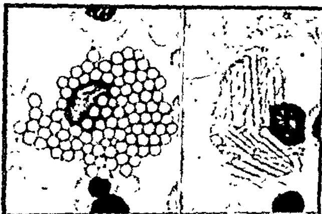
118 A. B
Varying degrees of athrocytosis (storage)
A. In a promonocyte
B in a senile monocyte, bone marrow



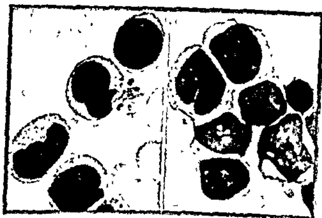
119. A B
Gaucher's disease. Monocytes, athrocytosis of lipoids:
A young monocyte, early athrocytosis,
B. older monocyte, advanced athrocytosis *Bone marrow.*



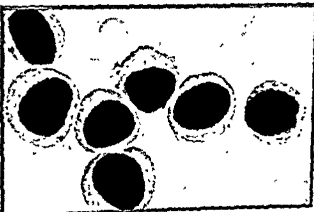
120. C D
C. and D the same Final stages of athrocytosis
("Gaucher's cells")



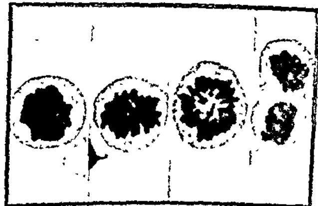
121. A B
Monocytes in the bone marrow exhibiting peculiar forms of athrocytosis A storage of spherical bodies,
B storage of crystal-like bodies



122. A B
Monocytosis
A in the blood in pneumococcal meningitis,
B in the bone marrow in syphilis



123. *Monocytic* leukaemia with predominance of monoblasts and promonocytes Blood.



124. A. B C D
Mitoses of monoblasts in the bone marrow
A and B prophase, C. metaphase D telophase

Plate 22

Leucocytes: Monocytes

(Continued)

Athrocytosis in the bone marrow; reactive and "primary" overproduction of monocytes; mitoses

Figures 119 and 120. Storage monocytes in Gaucher's disease in the bone marrow (Text on p 59). A. Mature monocyte showing early athrocytosis of cerebroside; the top right hand portion also shows phagocytosis Indented nucleus with loose structure B C and D Senile monocytes in terminal stages of athrocytosis The stored substances are colourless in C., pink in B and bluish in D Small, round, dry, atrophic nuclei Marked increase in cell volume, so-called "Gaucher's cells". In C. a group of blood platelets can be seen next to the nucleus, either superimposed on or beneath the cell

Gaucher's disease Bone marrow film Pappenheim staining (By courtesy of the University Clinic of Paediatrics, Zurich. Case reported by G Fanconi and C. Gasser [21])

Cerebroside is usually stored as a felt-like mass of needles, but is sometimes amorphous, as in the present case. In the two other storage diseases, Niemann-Pick's disease and Hand-Schüller-Christian's disease, amorphous storage is the rule.

Figure 121. Monocytes in the bone marrow exhibiting peculiar forms of athrocytosis (Text on p 59) A. Monocyte containing pale pink, spherical bodies of regular size; their origin is unknown but they are an occasional finding in bone marrow films B Monocyte containing peculiar pale blue, elongated crystal-like inclusion bodies, some of which are bent double Unique finding.

A Werlhof's disease. B Complete carrier of Pelger-Huët's anomaly Bone marrow films Pappenheim staining

Figure 122. Excessive production of monocytes (Text on p 60) A. Group of monocytes (chance accumulation). Top, monoblast with three indistinct nuclei Below, three almost mature monocytes and a group of blood platelets In reactive monocytosis monocytes, promonocytes and even monoblasts can be found in the circulating blood, cf Figure 108 D B Nest of monoblasts and promonocytes Indistinct nucleoli On the right, a normoblast.

A Pneumococcal meningitis in a child with a leucocyte count of 48,000 per cu mm., 3% being monocytes Nuclear shift of the neutrophils to the stage of promyelocytes Blood film Giemsa staining, B Syphilis with leucopenia Bone marrow film with 42% monocytes, 37% being monoblasts Pappenheim staining From the same case as Figures 127, 128 B and 128 C. (By courtesy of the University Medical Clinic, Basle)

Figure 123 Monocytic leukaemia with predominance of monoblasts and promonocytes (Text on p 60). The six cells on the left are young promonocytes Their nuclei have the typical, slightly blurred structure and contain several indistinct nucleoli. The cytoplasm is a mottled blue, with fine azurophilic granulation On the right, is a monocyte with an atypical, almost round nucleus



atypical neutrophils was ruled out by the fact that typical neutrophils in all stages of development were present and these gave a positive peroxidase reaction

In acute monocytic leukaemia the nuclei may not be indented, as they are in normal persons and in subacute cases of monocytic leukaemia.

Figure 124. Mitoses of monoblasts in the bone marrow (Text on p 58) A. and B Prophases, C. metaphase, D. telophase As in all other species of blood cell in mitosis, the cytoplasm has a mottled appearance, but it does not contain a true granulation. Blurred, relatively thick chromosomes, with obtuse angles

Agranulocytosis Bone marrow film with 26% monocytes, 6% neutrophils and 68% lymphocytes. Qualitatively normal monocytes Graham-Knoll peroxidase reaction. From the same case and preparation as Figures 54 and 107.

Plate 23

Leucocytes: Monocytes

(Concluded)

Mitoses and abnormal cells

Figure 125. Mitoses of promonocytes (Text on p. 58). A. Phagocytic promonocyte in prophase One of the inclusion bodies can be identified as an erythrocyte. This cell provides proof that even while still capable of undergoing division, the precursors of the monocytes may engage in phagocytosis B. Promonocyte in prophase. C. Large, pathological promonocyte in early prophase; the round chromatin borders of the nucleoli can still be seen. The chromosomes in all three cells have blurred outlines and are relatively thick, a feature characteristic of monocytes.

A. Anaemia Bone marrow film. Pappenheim staining B. Haemolytic disease of the new-born Blood film. Giemsa staining C. Monocytic leukaemia with predominance of monoblasts and promonocytes Blood film. Pappenheim staining From the same case as Figures 123, 227 E, 228 G, and 228 H.

Figure 126. Tetraploid monocytes with two diploid nuclei (twinning deformities) (Text on p. 59) The two separate nuclei in each monocyte can be readily distinguished.

A and B. Extensive pneumonia in the terminal phase. Blood film Giemsa staining C Cooley's disease. Bone marrow film Pappenheim staining.

Figure 127. Tetraploid monocytes in blood (Text on p 59) A. Promonocyte with two diploid nuclei (twinning deformity) B and C. Promonocytes with one tetraploid nucleus (monster cell)

Syphilis with neutropenia Blood film Graham-Knoll peroxidase reaction. From the same case as Figures 122 B, 128 B and 128 C

The characteristic features of immature monocytes are: negative peroxidase reaction, indistinct nuclear structure, invisible or poorly visible nucleoli, and very weak staining of the azurophilic granulation with Giemsa after the peroxidase reaction.

Figure 128. Polyploid monocytes (Text on p. 59). Tetraploid binuclear monoblast in metaphase (double monaster) Characteristic short, thick chromosomes with somewhat indistinct outlines B Polyploid monoblast in prophase The degree of polyploidy was impossible to determine (compare this large aggregate of chromosomes with the small one in the diploid mitosis in Figure 125 B) C Mononuclear, polyploid promonocyte, probably octoploid The nucleus is indistinct, and has a sponge-like structure with one vacuole The cytoplasm contains azurophilic granules

A. Healthy individual Bone marrow film Giemsa staining B und C from the same case as Figures 122 B and 127 Bone marrow film Pappenheim staining

Figure 129. Monocytes in lymph gland punctate in Boeck's disease (Text on p 59) A. above. monoblast with indented nucleus and two nucleoli. Below, denuded nucleus of a lymphocyte B above: large, atypical promonocyte Below normal monocyte C. Atypical monoblast with ruptured cytoplasm and partly fused nucleoli, a kind of "epithelioid cell".

Boeck's disease (lymphogranulomatosis benigna) Lymph gland punctate Pappenheim staining (By courtesy of Dr S J Leitner, Leysin)

Even in normal healthy persons monocytes in all stages of development are to be found not only in the bone marrow, but also in other organs, especially in the spleen and in the lymph glands.

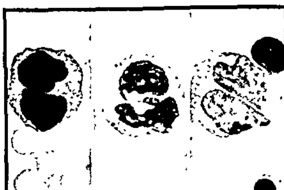
Figure 130. A Langhans' giant cell in lymph gland punctate in Boeck's disease (Text on p 59) Part of a Langhans giant cell The pigeon-blue cytoplasm and the patchy azurophilic granulation are evidence of the monocytic origin of this giant cell It must have developed either by a succession of mitoses in which the cytoplasm failed to divide, or by fusion of the liquefied cytoplasm of several cells The latter explanation is less probable Langhans' giant cells—like the epithelioid cells—are apparently monocytes which have undergone severe pathological changes due to toxic effects ("poisoned monocytes") From the same case and preparation as Figure 129

It is probable that the giant cells caused by foreign bodies and the giant cells seen in other infections (see Figure 255) are also of a fundamentally similar nature, namely giant polynuclear monocytes.

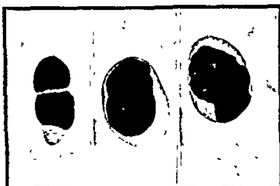
Magnification 1 1200



125. A B C
Promonocytes in prophase A with remains of ingested material, B in haemolytic disease of the new-born, C in promonocytic leukaemia



126 A B C.
Tetraploid monocytes with two diploid nuclei (twinning deformities) A, B in blood in terminal phase of pneumonia, C in bone marrow in Cooley's disease



127. A. B C
Tetraploid monocytes in the blood in syphilis
A with two diploid nuclei, B and C each containing one tetraploid nucleus



128 A B C
A Tetraploid monocyte in mitosis (double metaphase), B and C highly polyploid promonocytes in prophase and in interphase (resting phase)

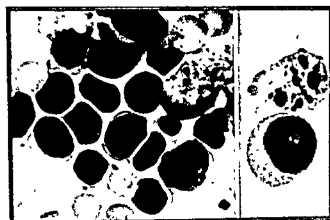


129 A. B C
Monocytes in lymph gland punctate in Boeck's disease (sarco dosis)
A. monoblast, B giant promonocyte C atypical monoblast

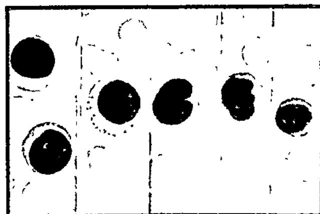


130 Langhans' giant cell (atypical polynuclear monocyte?) in lymph gland punctate in Boeck's disease

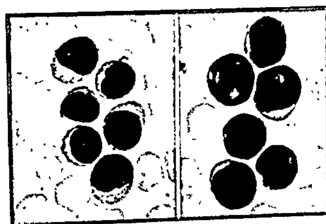
Magnification 1:1200



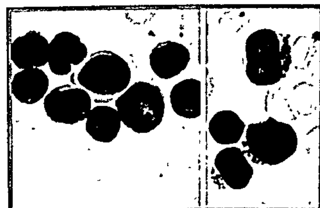
131. A B.
Lymphocytes: stages of development and a disintegrating (necrobiotic) cell



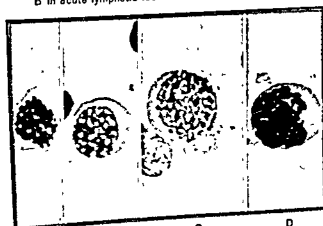
132. A B C. D. E
Mature lymphocytes in blood



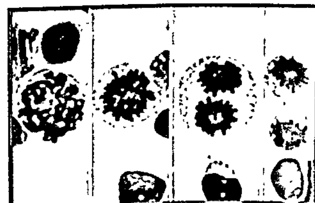
133. A. B.
Lymphocyte nests in bone marrow:
A in agranulocytosis,
B in acute lymphatic leukaemia.



134. A B
Lymphatic leukaemia A. lymphoblasts and lymphocytes in bone marrow, B lymphocytes with unusually coarse azurophilic granulation



135. A. B C. D
Mitoses of lymphoblasts
Prophases: A. in glandular fever, blood;
B to D in lymphadenosis, lymph gland punctate



136. E F G H
Lymphoblasts in lymphadenosis E prophase F metaphase G anaphase, H telophase Lymph gland punctate

Plate 24

Leucocytes: Lymphocytes

Stages of development; leukaemias; mitoses

Figure 131. Lymphocytes: stages of development (Text on p 61) A. Nest of lymphocytes Bottom right, a lymphoblast. The remaining cells are more or less mature lymphocytes No nucleoli are visible owing to the fact that the cells are lying relatively close together and even in lymphoblasts the nucleoli are usually covered Above, on the right, there is also a peroxidase-positive neutrophil, below which is a polychromatic normoblast. The small black square below on the right is a charcoal particle B below, lymphoblast with characteristic solitary nucleolus in a fine, close-mesh chromatin network Above: slightly crushed, necrobiotic lymphoblast (disintegrating form) The viscous basophilic chromatin is distributed throughout the thin oxyphilic chromatin in the form of isolated droplets, see also Figures 227–232

A. Child Normal bone marrow film Graham-Knoll peroxidase reaction Lymphocyte nests in healthy bone marrow are a regular finding in children and are occasionally seen in adults B Lymphatic leukaemia, film prepared from lymph gland punctate Pappenheim staining

Figure 132. Mature lymphocytes in blood (Text on p 61) A. to E.: normal lymphocytes The nuclei are dense and cloud-like with a coarse, lumpy, non-reticular structure The cytoplasm in B and D contains sharp, splinter-shaped azurophilic granules surrounded by small, pale haloes, which are characteristic of lymphocytes, cf the upper cell in Figure 44 D In C and D the nuclei have slightly indented outlines, so-called "Rieder forms". Such cells can also be found in small numbers in healthy persons, especially in children E. Particularly small lymphocyte, so-called "lymphatic plasma cell". Deep blue cytoplasm und pyknotic but not liquefied nucleus with coarse lumpy chromatin This cell is not a plasma cell, but a senile, degenerated lymphocyte such as is occasionally seen even in healthy persons, cf the lower cell in Figure 44 D

Blood films A and C to E. Giemsa staining B Pappenheim staining

Figure 133. Lymphocyte nests in the bone marrow. A. Mature specimens only with no nucleoli, including some Rieder forms. B Mainly lymphoblasts each with one nucleolus, only the lymphoblast at the bottom contains two nucleoli The cell above on the left is a pathological "blue" blood platelet.

A. Agranulocytosis Bone marrow film Graham-Knoll peroxidase reaction From the same case and preparation as Figures 54, 107, 124 B Acute lymphoblastic leukaemia Bone marrow film Graham-Knoll peroxidase reaction.

Figure 134. Two cases of lymphatic leukaemia. A. Lymphoblasts and lymphocytes in the bone marrow (Text on p 62) Two lymphoblasts surrounded by seven lymphocytes having almost denuded nuclei; some are Rieder forms

Chronic lymphatic leukaemia Bone marrow film. Pappenheim staining In lymphatic leukaemia, the bone marrow may contain more than 90% lymphocytes.

B. Lymphocytes with particularly coarse azurophilic granulation (Text on p 61) The cytoplasm of the lymphocytes contains peculiar, spherical azurophilic granules of various sizes Very rare finding In lymphatic leukaemia, the lymphocytes do not usually [illegible]

bodies, which usually occur singly They stain pink, like the erythrocytes, but do not contain haemoglobin since they give a negative Lepehne reaction

Figures 135 and 136. Mitoses of lymphoblasts (Text on p 61) A. to E. Prophases in various stages F Metaphase, G anaphase and H telophase The mitosis begins with a breaking up of the chromatin, during which phase the nucleoli can still be seen, as in B and C The chromosomes of the lymphoblasts are short and thick, their angles are obtuse, and they lie close together In G and H the cytoplasm is mottled in appearance cf Figures 32 and 37–40 In C and D., portions of the cytoplasm have become detached in the form of droplets during preparation of the film, owing to the fact that the cytoplasm of the lymphocytes does not press flat like that of other blood cells, but breaks up immediately, cf Figures 47 G., 234 D and 234 E.

A Lymphatic glandular fever Blood film Giemsa staining B to H. Lymphatic leukaemia Film prepared from lymph gland punctate Pappenheim staining (By courtesy of Dr S J Leitner, Leysin).

Plate 25

Leucocytes: Lymphocytes

(Concluded)

Atypical and deformed cells

Figure 137. Typical and atypical lymphocytes in lymphatic glandular fever (Text on p 61). **A. B.** and **C.**: three lymphocytes of normal size and four atypical lymphocytes of larger size than normal Some are ungranulated and the others contain azurophilic granules surrounded by characteristic haloes. No nucleoli are visible

Lymphatic glandular fever Blood film Giemsa staining

In lymphatic glandular fever, lymphocytosis is accompanied by monocytosis and plasmocytosis in the blood and by the presence of atypical forms, cf. Figure 187.

Figure 138. Polyploid lymphocytes (Text on p. 62) **A.** Tetraploid lymphocyte with two nuclei Both nuclei are normal in size **B.** Probably diploid lymphocyte with two nuclei. The small subsidiary nucleus is apparently derived from a small group of chromosomes split off during mitosis. **C.** Similar to A, but with a connecting filament; this does not indicate amitosis but is derived from a chromosome connecting bridge formed during the last atypical mitosis, cf Figures 40D. and 40E. **D.** Lymphocyte with four nuclei

Healthy person. Blood films. Giemsa staining.

Figure 139. Lymphocytes with atypical, partially heteroploid nuclei, from a case of severe whooping cough (Text on p 62) **A.** and **B** lymphocytes with severe reactive anomalies of the nucleus (polyploidy, hypodiploidy and Rieder forms) In A. below there *is also part of an immature neutrophil.*

Whooping cough in a child Final stage with a leucocyte count of more than 100,000 per cu mm. Blood film. Pappenheim staining (By courtesy of the Children's Hospital, Basle)

The pale blue "granules" on the left are due to **glass defects**, resulting from small air bubbles which have burst during manufacture of the slide. These glass defects retain staining solution and appear pale blue to dark violet in colour; when erythrocytes are superimposed on them, they may be mistaken for Howell-Jolly bodies or nuclei of erythroblasts Elongated glass defects of this nature have been described as micro-organisms and held to be the causative agent of cancer. (Text on p 48 [23]).

Figure 140. Lymphocytes with atypical, partially heteroploid nuclei, from a case of chronic lymphatic leukaemia (Text on *pp 61, 62) Lymphocytes with severely deformed, atypical nuclei, similar to those in Figure 139, but of "primary" origin* So-called "paralymphocytes", more correctly termed "atypical lymphocytes".

Lymphatic leukaemia in an old man with a leucocyte count of 219,200 per cu mm, 93 % being lymphocytes Blood film. Giemsa staining (By courtesy of the University Medical Clinic, Zurich)

Figure 141. Vacuolated atypical lymphoblasts in acute lymphatic leukaemia (Text on p 62) **A.** and **B**: Lymphoblasts with highly vacuolated cytoplasm and large grooved or indented nuclei The nucleolus is either invisible or very indistinct One of the cells in B is in prophase and shows a line of separation, it is probably the mitosis of a binuclear cell.

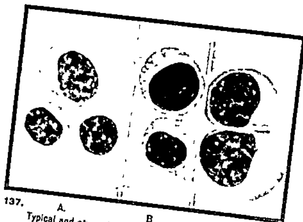
Acute lymphatic (lymphoblastic) leukaemia in a young child with a white blood count of 6,450—41,000 per cu mm., 94 % being lymphocytes Bone marrow film Giemsa staining (By courtesy of the Children's Hospital, Basle).

Figure 142 Atypical lymphoblasts (Text on p 62) **A.** and **B**: atypical lymphoblasts with almost denuded nuclei of irregular, glomerular appearance **C** and **D** mononuclear, polyploid (apparently octoploid) lymphocytes with nuclei of lobulated, wrinkled appearance, similar to those in A and B To be noted is the narrow border of cytoplasm in the cells in A. and B, although they are lying in the thin part of the film In A. above, there is also a peroxidase-positive neutrophil

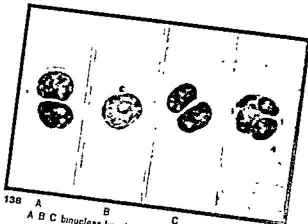
Acute aleukaemic lymphatic leukaemia in a small child with 80 % lymphocytes in the blood Blood film Graham-Knoll peroxidase reaction.

As Figures 131, 133 and 142 show, lymphocytes are always peroxidase negative.

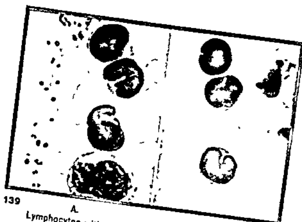
***From Figures 139 and 140 it is evident that a knowledge of the entire clinical picture, possibly even of the course* of the disease, is needed to decide whether qualitative and quantitative changes in the lymphocytes are reactive or leukaemic ("primary") in origin. The most conclusive evidence of the existence of leukaemia is provided by the presence of denuded cells and giant forms in the blood (see Figure 142), and by a high percentage of lymphocytes in the sternal marrow (see Figures 133B., 134A. and 141).**



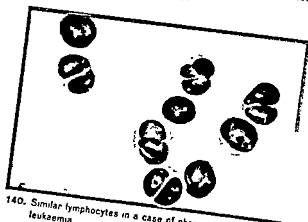
137. A. B C
Typical and atypical lymphocytes in lymphatic glandular fever



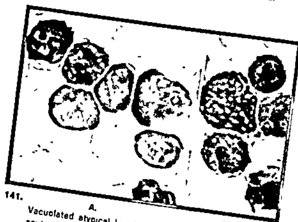
138 A B C D
A B C binuclear lymphocytes and D lymphocyte with four nuclei (polynuclear deformity)



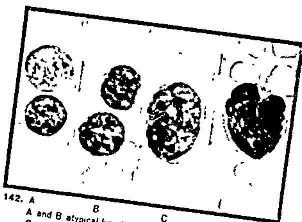
139 A. B
Lymphocytes with atypical, partly heteroploid nuclei in severe whooping cough



140. Similar lymphocytes in a case of chronic lymphatic leukaemia



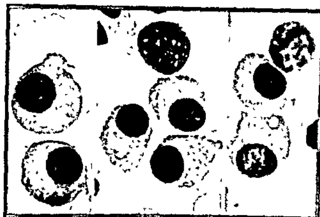
141. A. B
Vacuolated atypical lymphoblasts and atypical mitoses in acute lymphatic leukaemia Bone marrow



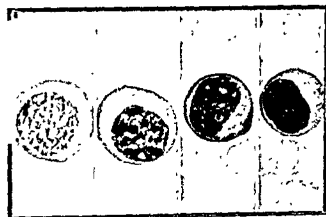
142. A B C D
A and B atypical lymphoblasts with almost denuded nuclei, C and D highly polyploid mononuclear lymphoblasts in acute lymphatic leukaemia Blood



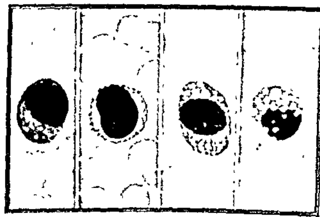
143. Plasma cells · stages of development
Plasmoblast, proplasmocytes, plasmocytes, bone marrow.



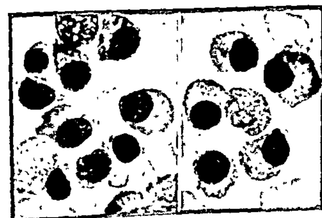
144. A B. C
Mature normal plasma cells in bone marrow.



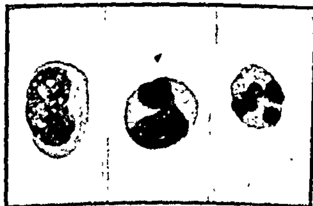
145. A. B C. D
Proplasmocytes in blood



146. A. B C D
Almost mature plasma cells in blood
some containing vacuoles



147. A. B
Nests of plasma cells in bone marrow A. in rubella,
B. in lymphogranuloma inguinale.



148. A B C
Atypical plasma cells A and B with indented nuclei
C. with segmented nucleus

Plate 26

Leucocytes: Plasma Cells

Stages of development; atypical cells

Figure 143. Plasma cells: stages of development (Text on pp 62, 63) Nest of normal plasma cells in proliferation Top left: plasmoblast with central nucleus and small, dark blue border of cytoplasm Below two proplasmocytes, the nucleus of the one on the right containing five nucleoli Above, two plasmocytes with eccentric nuclei, the one on the left containing a sharply defined vacuole, the one on the right wedged between the other cells — The cytoplasm has a particularly deep blue colour which masks the red component ("cyanophilia") In the middle are two monocytes with indefinite outlines and containing atrophic nuclei Such cells, which often contain stored material, can frequently be found as "regional monocytes" in the centres of formation of various species of blood cell, especially in those of the erythrocytes, cf Figures 147, 179, 180 (Text on p 59 [24])
Anaemia in an adult. Bone marrow film Giemsa staining

Figure 144 Mature normal plasma cells in the bone marrow (Text on p 63) A B and C.: eccentric nuclei with so-called "cart-wheel structure" and in direct contact with the zone of pale archoplasm The cytoplasm of some of the cells contains vacuoles In a few cells, small particles of cytoplasm have become detached In B and C. above, crushed nuclei (Gumprecht's shadows) are also visible
Two healthy adults Bone marrow films A. Giemsa staining, B and C Pappenheim staining

Figures 145 and 146. Proplasmocytes in blood (Text on p 63) Proplasmocytes and almost mature plasmocytes, some of which contain prematurely pyknotic nuclei In Figures 145 A, and 145 C nucleoli are clearly visible Figure 146 C. shows very coarse, lumpy chromatin, so-called "cart-wheel structure". The vacuolation of the cells is indistinct in Figures 145 A. and 145 B, distinct but fine in Figures 146 A and 146 B, and coarse in Figures 146 C and 146 D The contents of the vacuoles have a pale reddish-blue colour
Healthy persons and patients without qualitative changes in the plasma cells 146 B Pappenheim staining, otherwise Giemsa staining

Mature plasmocytes can seldom be found in the blood. Usually, it is the proplasmocytes which are released into the blood stream.

Figure 147. Nests of plasma cells in the bone marrow (Text on pp 63, 64) A. Nest containing five plasma cells Nearby are four polychromatic normoblasts and portions of peroxidase-positive leucocytes Above: part of a regional monocyte B Five mature plasma cells surrounding a regional monocyte
A. Rubella Bone marrow film Graham-Knoll peroxidase reaction. (By courtesy of the University Medical Clinic, Zurich) B Lymphogranuloma inguinale (Nicolas-Favre's disease) Bone marrow film Pappenheim staining (By courtesy of the University Medical Clinic, Zurich)

Nests of plasma cells in the bone marrow are more common in patients suffering from rubella than in healthy persons. The relative plasma cell count in the bone marrow picture is not necessarily higher, however, as it may happen that no nests are included in the count. In lymphogranuloma inguinale, however, the count always reveals a marked increase in the number of plasma cells.

Figure 148. Atypical plasma cells (Text on p 63) A. Proplasmocyte with indented nucleus and three nucleoli, one of which is very large B Proplasmocyte with indented nucleus C Proplasmocyte with segmented nucleus (five segments) The cytoplasm is vacuolated in all these cells Normally, the nuclei of the plasma cells are not segmented
A Healthy individual. Blood film Giemsa staining B Agranulocytosis Bone marrow film. Graham-Knoll peroxidase reaction C Plasmocytoma Bone marrow film Pappenheim staining From the same case as Figure 154 B (By courtesy of the University Medical Clinic, Zurich).

Plate 27

Leucocytes: Plasma Cells

(continued)

Atypical cells; leukaemia; plasmocytoma

Figure 149. Atypical plasma cells (Text on p. 63). A. and B Plasma cells with pronounced red component ("flaming" plasma cells). Occasional finding in various diseases C. Normal, well spread out, vacuolated plasma cell for comparison As a rule, the red component of the cytoplasm is scarcely visible in normal cells, even when they are well spread out

A. and B. Essential panmyelopathy. From the same case as Figure 188. (By courtesy of the University Medical Clinic, Zurich) C. Myelosis. Bone marrow films Pappenheim staining

Figure 150. Plasma cells in the blood in plasmocytoma with plasma cell leukaemia (Text on p. 64) A. Plasmoblast with large nucleus. In the centre of the nucleus, near the vacuole, is a group of indistinct nucleoli The border of cytoplasm is narrow and vacuolated B Two proplasmocytes containing several nucleoli. In the centre is a segmented neutrophil C. Three almost mature plasma cells, some of which contain small vacuoles.

A. and B. Acute proplasmocytic leukaemia with some plasmoblasts Blood film Pappenheim staining (By courtesy of the University Medical Clinic, Lausanne). C. Chronic plasma cell leukaemia Blood film. Graham-Knoll peroxidase reaction (By courtesy of the University Medical Clinic, Basle).

Figure 151. Plasmocytoma, so-called "multiple myeloma" (Text on p 64) Nest of fairly mature plasma cells One is binuclear and another contains two vacuoles, while two cells have been compressed. The pale archoplasm is in direct contact with the nucleus, in contrast to that of the osteoblasts which lies at some distance from the nucleus, see Figures 201, 202. The outlines of the cells are sharply defined The spaces between the cells result from the shrinkage which takes place on drying To be noted is the typical oyster-shell form of some of the cells.

Plasmocytoma without plasma cell leukaemia (the more common form) Bone marrow film. Pappenheim staining (By courtesy of the University Medical Clinic, Basle)

Figure 152. Special elements present in plasmocytoma (Text on p. 63). A. Well spread out plasma cells containing numerous vacuoles, some of which are very large The bluish colour of the vacuole contents indicates the presence of partly condensed protein-like substances. B So-called Russell corpuscles formed by fusion of the contents of vacuoles of destroyed plasma cells, cf. Figure 48 B. The reddish-violet filamentous structure on the left is a precipitate of stain

Plasmocytoma Bone marrow films. Pappenheim staining. (B by courtesy of Dr. S J Leitner, Leysin)

Figure 153. Two cases of plasmocytoma with inclusion bodies in the plasma cells (Text on p 63). **A. Crystalline inclusion bodies.** The cytoplasm contains sharply defined azurophilic needles, similar in appearance to Auer rods One of the cells is in prophase Near the lower cell is a small detached fragment of cytoplasm.

Plasmocytoma with plasma cell leukaemia Bone marrow film. Pappenheim staining (By courtesy of Dr. B Steinmann, Bern [25]).

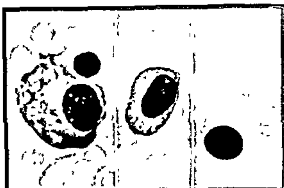
B. Snapper-Schneid inclusion bodies. These inclusion bodies form during treatment with amidines (stilbamidine, pentamidine) and consist of amidine and ribonucleic acid They are purely basophilic granules of various sizes [26]

Patient with plasmocytoma after stilbamidine treatment. Pappenheim staining (By courtesy of the University Medical Clinic, Freiburg, Germany)

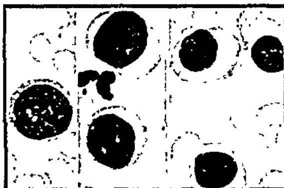
Figure 154. Plasma cells with segmented nuclei in plasmocytoma (Text on p 63). A. and B Plasma cells with highly segmented nuclei and vacuolated cytoplasm. The club-like shape of the segments and their rosette formation indicate that they are not the result of a normal segmentation process but are due to mitosis having been arrested in metaphase This may have been caused by some metabolic product or medicament which acted as a mitotic poison.

A. Plasmocytoma with numerous segmented plasma cells B Plasmocytoma with fewer segmented plasma cells From the same case as Figure 148 C. Bone marrow films. Pappenheim staining (By courtesy of the University Medical Clinic Zurich)

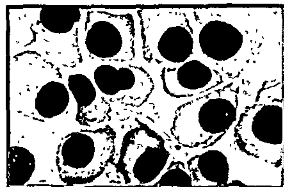
Magnification 1 1200



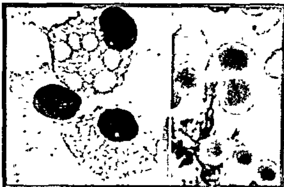
149 A. B C.
A. and B "flaming" appearance of plasma cells due to atypical red staining C well spread out normal plasma cell



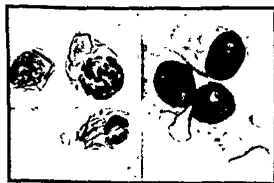
150. A B C
A and B proplasmocytic leukaemia with some plasmoblasts, C plasma cell leukaemia Blood



151. Plasmocytoma
plasma cells in the bone marrow



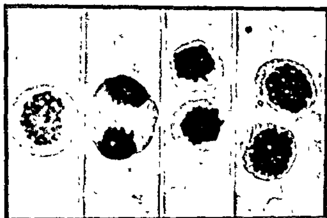
152. A B
Plasmocytomas
A with highly vacuolated plasma cells
B with extracellular Russell corpuscles Bone marrow



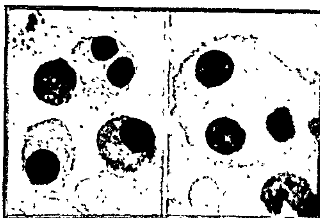
153 A. B
Plasma cells from plasmocytomas in the bone marrow
A. with crystalline inclusion bodies B with Snapper-Schneid s inclusion bodies after stilbamidine treatment.



154. A. B
Plasma cells with segmented nuclei in plasmocytoma
Bone marrow



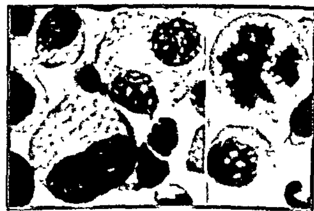
155. A. B. C D.
Mitoses of plasma cells in the blood:
A. prophase, B anaphase,
C. and D nuclear unrest immediately after division



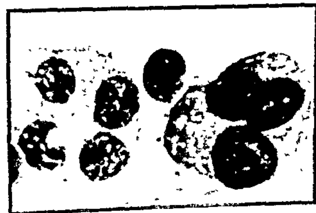
156. A. B
Plasma cells in normal bone marrow: A tetraploid cell with two diploid nuclei (twinning deformity),
B hexaploid (?) cell with three diploid nuclei



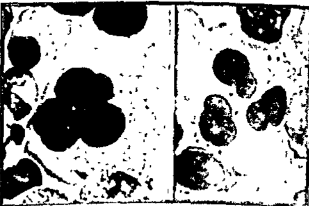
157. A B
Plasma cells in bone marrow A diploid cell in metaphase and tetraploid cell with two diploid nuclei (twinning deformity).
B atypical prophase.



158. A B
Plasma cells in plasmocytoma A. tetraploid, with two diploid nuclei (twinning deformity) and with single tetraploid nucleus (monster); B multipolar mitosis



159. Octoploid plasma cells in plasmocytoma:
left, with four diploid nuclei, right, with one tetraploid and two diploid nuclei.



160. A B
Highly polyploid multinuclear plasma cell with atypical nuclei Plasmocytoma

Plate 28

Leucocytes: Plasma Cells

(Concluded)

Mitoses and deformed cells

Figure 155. Mitoses of plasma cells in blood (Text on p 63) A. Prophase, B anaphase, C and D plasma cells shortly after a completed telophase with nuclear unrest still apparent In B the cytoplasm contains a few indistinct vacuoles, while in C they are more distinct. The two daughter cells in C and D must have completed their separation in the wet film, otherwise they would not be lying side by side This indicates that the blood does not inhibit mitosis

A. and B healthy individuals, C. lymphatic glandular fever, D chronic intestinal affection Blood films A. and B Giemsa staining C and D Pappenheim staining.

A. and B. show that mitosis of plasma cells occasionally takes place in the blood even in healthy persons. Except under pathological conditions, the lymphocyte is the only other species of blood cell which can enter the blood stream in a stage still capable of proliferation, but this, too, is an extremely rare finding.

Figure 156 Polyploid plasma cells in normal bone marrow (Text on p 63) A. Above tetraploid plasma cell with two diploid nuclei (twinning deformity) Below: two normal (diploid) plasma cells and a nuclear shadow B Hexaploid plasma cell with three diploid nuclei, it is probable that this cell was originally octoploid with four nuclei but that a portion containing a nucleus has been detached

Individuals with normal bone marrow Bone marrow films A Pappenheim staining B Giemsa staining

Figure 157. Mitoses of plasma cells in bone marrow (Text on p 63) A. Below: normal metaphase of a proplasmocyte Above: tetraploid plasma cell with two diploid nuclei B Below: atypical prophase of a polyploid proplasmocyte Above large, pathological plasmocyte

A. Individual with normal bone marrow B Plasmocytoma Bone marrow films A. Giemsa staining. B Pappenheim staining B From the same case as Figure 160 (By courtesy of the University Medical Clinic, Basle)

Figures 158 and 159. Polyploid plasma cells in sternal marrow in plasmocytoma (Text on p 63) **Figure 158: A.** Two tetraploid plasma cells, the upper with two diploid nuclei (twinning deformity), the lower cell with four diploid nuclei (monster), B tetrapolar telophase of a polyploid plasma cell. **Figure 159:** on the left is an octoploid plasma cell with four diploid nuclei, formed as the result of a tetrapolar telophase as in Figure 158 B.; on the right, an octoploid plasma cell with one tetraploid and two diploid nuclei (see Figure 105 B) A number of vacuoles can be more or less clearly distinguished in the cytoplasm Diploid plasma cells are present in Figure 158 A, top left, and Figure 158 B below In the upper cell in B, the paramitotic mottled appearance of the cytoplasm is clearly evident

Patient with plasmocytoma Sternal marrow Pappenheim staining (By courtesy of the 2nd Medical Clinic of the University, Basle)

Figure 160. Highly polyploid plasma cells with several nuclei in plasmocytoma (Text on p 63) Large juvenile polyploid plasma cells A Cell with six overlapping nuclei of nearly equal sizes (12-ploid), the nuclei are probably joined in pairs B Cell with six nuclei, which are fused in pairs of unequal size, giving them a skittle-like appearance. These pairs have been formed by atypical mitoses owing to unequal division of the chromosomes and the formation of chromosome bridges

Plasmocytoma Bone marrow film Pappenheim staining

Figures 174 A., 146 B. and 150 C. show that plasma cells are peroxidase negative.

Plate 29

Leucocytes: Megakaryocyte-platelet system

Stages of development

Figures 161 to 165. Megakaryocytes: stages of development (Text on pp 64, 65).

A. and B. **Diploid megakaryoblasts.** The cells are relatively large, the structure of the nucleus is vague and the nucleoli are indistinct. C Multipolar mitosis of a megakaryoblast.

D and E. **Tetraploid megakaryoblasts.** The cell in D has two diploid nuclei, the one in E. has a single tetraploid nucleus The round hole in the nucleus in E. is filled with cytoplasm and is not a nucleolus.

F. and G. **Octoploid megakaryoblasts.** The cell in F. has four diploid nuclei, the one in G has a single octoploid nucleus.

H. I and K. **Endomitoses or fused mitoses.** H. shows a polyploid megakaryoblast in metaphase, I. a promegakaryocyte in prophase, and K. a promegakaryocyte in metaphase.

L. **Promegakaryocyte in interphase (resting stage)** The cytoplasm has already become partially acidophilic or polychromatic. This distinguishes the promegakaryocytes from the megakaryoblasts in which the cytoplasm is still completely basophilic The transition from megakaryoblast to promegakaryocyte is not linked with any particular degree of polyploidy, for the oxyphilic component can appear at an early or at a late stage of development.

To be noted are the very characteristic cytoplasmic projections and detached fragments of cytoplasm to be seen in A. B. D E. F. I K. and L. They indicate the extraordinary fragility of these cells, a property connected with the formation of blood platelets, of which they are the parent elements Other large cells may also develop cytoplasmic projections (see Figure 36 E.), but not with the same regularity The cell in K. contains a rosette of vacuoles, and is bordered above by partly agglutinated blood platelets Normoblasts are present in G and H

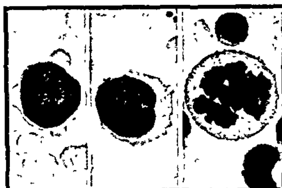
A. Chronic myelogenous leukaemia B. Normal bone marrow C D E. F. H I and L. Werlhof's disease; the number of megakaryocytes is greatly increased but their appearance is normal G Untreated pernicious anaemia K. Gaucher's disease A. Blood film B. to L. marrow films A B. and G. Giemsa staining. C. to F. and H to L. Pappenheim staining

All the elements are normal in appearance.

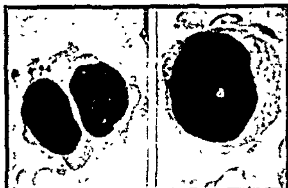
Figure 166. Part of a normal, fully mature megakaryocyte (Text on p 65) Giant cell: with the magnification here employed only part of the cytoplasm could be shown Dense, irregular, lumpy nucleus; oxyphilic cytoplasm with fine azurophilic granulation. Top right: compressed erythrocytes coloured green as the result of the Lepehne reaction The megakaryocyte only takes up the subsequent Giemsa stain

Bone marrow film from an individual with cells of normal appearance Lepehne peroxidase reaction.

Magnification 1:1200



161. A B C.
Megakaryocytes stages of development
A and B diploid megakaryoblasts, C mitosis



162. D E.
Megakaryocytes stages of development
Tetraploid megakaryoblasts: D with two diploid nuclei,
E. with one tetraploid nucleus Bone marrow



163 F G.
Megakaryocytes stages of development.
Octoploid megakaryoblasts F with four diploid nuclei
G with one octoploid nucleus Bone marrow



164. H I
Megakaryocytes stages of development.
Endomitoses H polyploid megakaryoblast in metaphase
I promegakaryocyte in prophase



165 K L.
Megakaryocytes stages of development
Promegakaryocytes K in endomitotic metaphase,
L. resting stage



166 Normal fully mature megakaryocyte Bone marrow

Magnification 1:450, 1:600 and 1:1200

1:1200



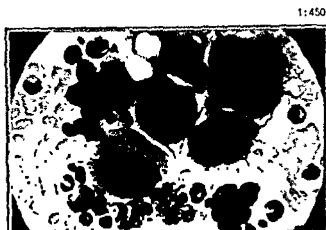
167. Pathological "hyaline" promegakaryocyte. Bone marrow

1:600



168. A. B.
A promegakaryocytes and B megakaryocyte *with nuclear* hypersegmentation in pernicious anaemia

1:450



169. Essential thrombocytopenia
Overproduction of megakaryocytes in the bone marrow

1:600



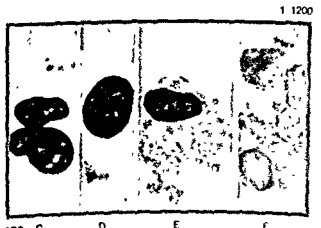
170. Megakaryocytic leukaemia
Overproduction of megakaryocytes and platelets
Bone marrow.

1:1200



171. A. B.
A. pathological promegakaryocyte and B. pathological megakaryocyte containing numerous small nuclei
Bone marrow

1:1200



172 C. D. E. F.
Small nuclei from pathological megakaryocytes in the blood
Same case as A. and B.

Plate 30

Leucocytes: Megakaryocyte-platelet system

(Continued)

Atypical megakaryocytes; reactive overproduction of megakaryocytes

In Figures 168 and 170 a magnification of only 1 600 has been used, in Figure 169 a magnification of 1 450

Figure 167. Pathological "hyaline" promegakaryocyte (Text on p 65) Relatively loose nuclear structure. The cytoplasm is basophilic and clear blue in colour, azurophilic granules being present only in the indentation of the nucleus A rosette of vacuoles surrounds the granules At this stage of development, the cytoplasm is normally almost completely oxyphilic, pink in colour, and contains azurophilic granules, as in Figure 165

Patient suffering from aplastic anaemia Bone marrow Pappenheim staining

Figure 168. Promegakaryocytes and a megakaryocyte with nuclear hypersegmentation in pernicious anaemia (Text on p 65) A and B Two promegakaryocytes and a megakaryocyte with hypersegmented nuclei Some of the nuclear segments have become detached from the highly ramified nuclear mass The unequal size of these segments distinguishes them from osteoclast nuclei which are all equal in size, cf Figures 203 to 205

Pernicious anaemia, treated cases Bone marrow films. Pappenheim staining

In pernicious anaemia, not only the neutrophils (Figure 81), eosinophils (Figure 64 C) and basophils but also the megakaryocytes have highly segmented nuclei, even in treated cases.

Figure 169. Reactive overproduction of megakaryocytes in the bone marrow in essential thrombocytopenia (Text on p 66). Nest of six megakaryocytes The nuclei are partly covered by a thick layer of granulated cytoplasm This ill-defined appearance of the nucleus in thick parts of the film should not be taken as evidence of the passage of intact nuclear substance into the cytoplasm, since when megakaryocytes are spread out thinly the nuclei are sharply defined No platelets are visible

Essential thrombocytopenia (Werlhof's disease) Bone marrow film Pappenheim staining (By courtesy of the University Medical Clinic, Zurich)

Figure 170. Megakaryocytic leukaemia (Text on p 66) On the left are three promegakaryocytes with pathological blue cytoplasm The cells are agglutinated with a large mass of platelets lying to the right A denuded necrobiotic nucleus from a megakaryocyte and a few stray leucocytes are to be seen in the midst of the platelets

Megakaryocytic leukaemia characterized by marked overproduction of megakaryocytes in the bone marrow and by increase in the number of platelets in the blood up to 3 000 000 per cu mm (By courtesy of the University Medical Clinic, Lausanne, case published by G Hemmeler [27])

Figures 171 and 172. Pathological megakaryocytes containing numerous nuclei; passage of nuclei into the blood stream (Text on p 65) A. Promegakaryocyte containing eight nuclei, six of which partially cover one another The nuclei are probably diploid. B Megakaryocyte containing 12 approximately diploid nuclei of unequal size C to F Megakaryocyte nuclei which have been released into the blood stream In C, and D the nuclei are lying free, while in E and F they are agglutinated with blood platelets Characteristic dark-coloured, dense chromatin, Lying above the megakaryocyte in B is an erythrocyte which has turned green due to the Lepehne reaction, similarly coloured erythrocytes are to be seen on the left of the picture

When a megakaryocyte develops normally a cell with a single giant nucleus results owing to endomitosis or fused mitoses. In the pathological megakaryocytes shown here however the nuclei have continued to divide mitotically right up to the mature stage, in the same manner as osteoclast nuclei normally do (cf Figures 203 to 205) The mature polynuclear megakaryocytes may be distinguished from osteoclasts by the density of the chromatin, the absence of nucleoli and the fact that the nuclei are often unequal in size and may be joined by bridges Moreover, some of the megakaryocytes in such cases have normal nuclei, and all intermediate forms between these and the polynuclear cells are present. The nuclei of the polynuclear promegakaryocytes may be distinguished from osteoclast nuclei by the fact that they contain several small nucleoli, although these are often concealed Osteoclast nuclei generally have only a single nucleolus which is relatively large and clearly visible (Figures 203 204) The nuclei of the polynuclear megakaryocytes are able to gain entrance to the peripheral blood owing to the fact that they are small enough to pass through the lung capillaries, whereas normal megakaryocyte nuclei are too large to do this The production of polynuclear megakaryocytes has been studied mainly by Kabelitz [28]

Man aged 60 with marked cardiac congestion Bone marrow film. Lepehne reaction. (By courtesy of Prof A. Alder Aarau).

Plate 31

Leucocytes: Megakaryocyte-platelet system

(concluded)

Platelets; fragments of megakaryocyte nuclei; twinning deformity

In Figure 178 a magnification of only 1:600 has been used

Figure 173. Normal blood platelets (Text on p 65) A. B and C. Normal blood platelets, lying singly or in small groups, some superimposed on normocytes. The platelets have a granular structure and their outlines are not sharply defined When superimposed on normocytes, they are surrounded by haloes. The normocyte in C. below contains one Howell-Jolly body. Howell-Jolly bodies are homogeneous, have sharply defined outlines and are not surrounded by haloes.

A. and B. Healthy individuals. C. Fibrosis of the spleen. Blood films. Giemsa staining

Figure 174. Agglutinated blood platelets (Text on p 67) The individual platelets can be distinguished by the small groups of granules. Their hyalomere has coalesced to form a coherent mass This is a common finding in the end portions of blood films when there has been a delay in their preparation, while in bone marrow films, owing to the time taken in carrying out marrow puncture, this early coagulation phenomenon is always seen, provided that no haemorrhagic diathesis is present. In bone marrow films, therefore, the mass of agglutinated blood platelets often contains megakaryoblasts, promegakaryocytes megakaryocytes or fragments of these cells, since all the stages of development in this system possess the characteristic property of agglutinating with one another [3]

Individual with normal blood platelets Blood film. Giemsa staining

Figure 175. Blood platelets under special conditions (Text on pp 66, 67) A Blood platelets with adherent fibrin needles. These needles have sharp outlines which distinguish them from the irregular cytoplasmic processes. Fibrin needles can be seen in deeply stained films prepared from coagulating blood In general, fibrin is chromophobic towards Pappenheim stain and takes on only a pale pink colour. B Pathological blood platelets of relatively large size The two lower ones have normal granulation the two in the centre are only sparsely granulated, and the one above on the left is ungranulated. Ungranulated blood platelets are distinguished from erythrocytes by their pure pink colour, free from any tinge of yellow

A. Individual without haematological changes B Myelogenous leukaemia Blood films Giemsa staining.

Figure 176. Giant platelets and fragments of megakaryocyte nuclei in blood (Text on p 66). A to C Large fragments of cytoplasm from megakaryocytes, so-called "giant platelets". They have failed to divide into platelets of normal size D Pathological platelet with blue hyalomere, so-called "blue platelet", cf. Figure 133 B E. Very dense fragment of a necrobiotic megakaryocyte nucleus which has found its way by chance into the circulating blood

A. and D Chronic myelogenous leukaemia. B., C. and E Healthy individuals Blood films. A. B. C E. Giemsa staining D. Pappenheim staining.

Figure 177. Fragments of megakaryocyte nuclei in blood (Text on p 66). A B C. and D Dense fragments of megakaryocyte nuclei, or, possibly small nuclei from polynuclear megakaryocytes In A. and B they are agglutinated with numerous platelets, in C. only with a few platelets.

A. Pneumococcal meningitis. From the same case as Figure 122 A. B and D. Chronic myelogenous leukaemia C Endocarditis lenta Blood films A. Giemsa staining B C. D. Pappenheim staining.

Fragments of megakaryocyte nuclei can often be found in the circulating blood in chronic myelogenous leukaemia, and occasionally in other diseases and in healthy individuals. They agglutinate easily with platelets.

Figure 178. "Twinning deformity" of a megakaryocyte (Text on p 23). A Binuclear megakaryocyte Two fully mature, polyploid nuclei of irregular shape, almost mirror images of each other, in a common cytoplasm Both nuclei are normal in size B A well spread out. normal megakaryocyte with a single nucleus for comparison.

A. Untreated pernicious anaemia. B. Healthy individual. Bone marrow films. Giemsa staining

Magnification 1:600 and 1:1200

1:1200



173 A B C
Scattered blood platelets. In C an erythrocyte with Howell-Jolly body is also present. Blood

1:1200



174. Agglutinated blood platelets in blood film

1:1200



175 A B
Blood platelets
A with fibrin needles due to advanced coagulation
B pathological forms

1:1200



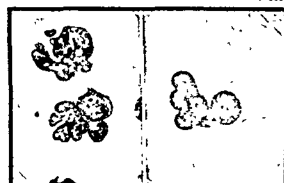
176. A B C D E
A to D undivided fragments of cytoplasm (giant platelets), D being a "blue platelet". E fragment of a megakaryocyte nucleus. Blood

1:1200



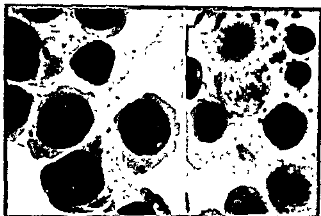
177 A B C D
A, B, C fragments of megakaryocyte nuclei agglutinated with platelets in the blood, D naked fragment of a megakaryocyte nucleus in blood

1:600



178 A B
A giant megakaryocyte with two nuclei (twinning deformity)
B normal mononuclear megakaryocyte for comparison

Magnification 1:1200



179. A B

Regional monocytes:
A. In a megaloblast nest, B In a normoblast nest.
Bone marrow.



180. C. D.

Regional monocytes in normoblast nests.
C. with necrobiotic nucleus,
D with necrotic nucleus Bone marrow



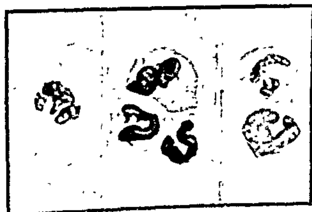
181. A. B C D

Alder's anomaly profuse azurophilic granulation of the leucocytes Pappenheim staining



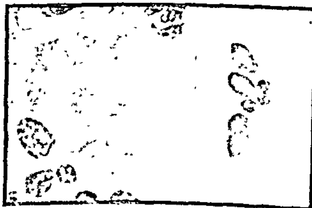
182. E F G H

Alder's anomaly E to G Pappenheim staining
H modified peroxidase reaction for eosinophils



183. A. B C

May Hegglin's anomaly polyphilic disorder of maturation
Doehle's inclusion bodies in leucocytes Blood



184. D

May Hegglin's anomaly D [illegible]
megakaryocyte showing [illegible]
granulomere bone marrow [illegible]

Plate 32

Leucocytes: Regional monocytes of the haemopoietic nests Anomalies affecting several species of blood cell simultaneously

Figures 179 and 180. Regional monocytes in haemopoietic nests (Text on p 59, see also Figures 143, 147) **A.** In a megaloblast nest. The regional monocyte may be seen towards the top of the picture in the centre. It has a small, mature nucleus and its cytoplasm is extended in long, tentacle-like processes which envelop the surrounding megaloblasts and even appear to penetrate inside them, as in the case of the cell below. **B** In a normoblast nest. The regional monocyte is spread out very thinly and occupies almost the entire visual field. The green mass at the top consists of ingested peroxidase-positive material. Below this is the nucleus, which is poor in chromatin and indented. Below the nucleus is a pale violet mass of ingested material. On the right, are a few necrobiotic nuclei and other debris from cells which have been consumed. On the left and below may be seen a few normoblasts from the large nest, in the centre of which the regional monocyte is lying. **C.** and **D** In normoblast nests. In C. the regional monocyte is in the centre. The nucleus is necrobiotic and liquefied, a sign that the cell is dying. Below are two intact promonocytes. Above are some normoblasts from the haemopoietic centre in which these monocytes are situated. In D a regional monocyte is lying in the centre of a nest of normoblasts. The cell is necrotic and the nucleus completely dissipated, destruction having progressed farther than in C. The streaks in the cytoplasm are caused by the remains of ingested material [24]

A Untreated pernicious anaemia. Giemsa staining. B C D Cooley's disease. B Graham-Knoll peroxidase reaction, C. and D. Pappenheim staining. Bone marrow films. (B C. and D. by courtesy of the Children's Hospital, Basle).

Figures 181 and 182. Alder's anomaly of leucocyte granulation (Text on p. 16) Unusually profuse azurophilic granulation of all leucocytes with the exception of the plasma cells. Mature stages. Hereditary anomaly. **A.** Above, a monocyte, below, a basophil. **B** and **C** Above two eosinophils. The granules are violet to greenish-gray instead of red, this is a typical finding. Below, two neutrophils. **D** Above a monocyte. Below two neutrophils. **E.** Below: a neutrophil. Above lymphocyte without granulation. **F.** Lymphocyte with profuse granulation. **G** Vacuolated plasma cell without granulation. **H** Above: peroxidase-positive eosinophil. The abnormal violet granules have remained. The other cells are a peroxidase-negative neutrophil and a monocyte.

All the basophils eosinophils and neutrophils, most of the monocytes, and a smaller fraction of the lymphocytes contain abnormal granulation. The basophils can be clearly differentiated from the eosinophils by means of the peroxidase reaction or staining with toluidine blue. The nuclei of many neutrophils are pale in colour and covered by dense granulation, an appearance not seen under normal conditions.

Alder's anomaly in a ten year old girl. Blood films. A. to G Pappenheim staining. H Modified peroxidase reaction for eosinophils. (By courtesy of Prof A. Alder, Aarau [29])

Figures 183 and 184. Hegglin's (May-Hegglin's) anomaly or hereditary polyphylic disorder of maturation (Text on p 16) **A.** Basophil. **B** Above eosinophil. Below two neutrophils. **C** Above peroxidase-positive neutrophil. Below monocyte. In all the cells, the cytoplasm contains blue inclusion bodies. The specific granulation of the basophil has been washed out, with the result that the blue patches have become visible. **D** Detached fragment of cytoplasm from a megakaryocyte. The unusually large size of the patches of granulomere indicates the future formation of giant platelets. **E.** Giant platelets in the blood.

May-Hegglin's anomaly in a man aged 50. A. B C and E. Blood films. D Bone marrow film. A and C Graham-Knoll peroxidase reaction. B and D Pappenheim staining. E. Giemsa staining. (By courtesy of Dr. R. Hegglin, Zurich [30]).

Plate 33

Leucocytes: Lupus erythematosus cells Simultaneous overproduction of different species

Figures 185 and 186. *Phagocytic leucocytes in lupus erythematosus - so-called "L. E. cells" in the bone marrow* (Text on p 18) A, B and C Phagocytic *neutrophils*. A. Three juvenile neutrophils in one of which the cytoplasm contains ingested, necrotic nuclear remains. B. *Segmented neutrophil and juvenile neutrophil* containing ingested nuclear material C. above: necrotic nucleus surrounded by three segmented neutrophils. D. above and E. centre: monocytes containing ingested nuclear material F. Centre: single, disintegrating neutrophil containing a homogeneous, necrotic nucleus and with a border of granulated cytoplasm. G. Crushed necrotic nucleus extruded from a phagocytic neutrophil. In D below there is also a monocyte, and in E. below and F. below there are a few plasmocytes

Woman aged 43 suffering from acute disseminated lupus erythematosus. Bone marrow film. Leishman staining (Pappenheim staining using specially prepared May-Grünwald solution). (By courtesy of Prof. L. Berman, Detroit [31])

The amorphous masses must be nuclear substance, since the Feulgen reaction is positive. In patients suffering from lupus erythematosus, such necrotic nuclei may be found anywhere in the body fluids and tissues.

Blood plasma taken from erythematosus patients may give rise to similar disintegrating forms in the blood and tissue fluids of healthy individuals. The process of destruction is not quite the same as that normally observed, cf. Figures, 227 and 232. The nuclei do not liquefy and become homogeneous, but form a coherent, felt-like mass, as can be seen in crushed specimens (Figure 186 G) Unlike ordinary necrotic nuclei, which behave like drops of mercury, these nuclei do not break up into small droplets. Moreover, they do not stain so deeply L. E. cells were first described by Hargraves, Richmond and Morton [32]

Figure 187. Lymphatic glandular *fever* (Text on pp 60, 62). A. Above: a monocyte Below: a staff neutrophil and a plasma cell (proplasmocyte) with vacuoles B Above: lymphocyte with indented nucleus. Below: a proplasmocyte C. Two lymphocytes with characteristic haloes surrounding the azurophilic granules The upper lymphocyte is abnormally large

Lymphatic glandular fever (Infectious mononucleosis) in a child Blood film. Giemsa staining From the same case as Figure 137.

In lymphatic glandular fever there is overproduction and often deformation of the blood cells which are produced in the lymphatic tissue, i.e., the lymphocytes, monocytes and plasma cells The blood picture therefore presents many different forms and is said to be "variegated" in contrast to the monotonous blood picture, containing mainly lymphocytes, found in lymphatic leukaemia and infectious lymphocytosis.

Figure 188. Bone marrow film in essential panmyelopathy (Text on pp. 14, 60) A Two monocytes surrounded by lymphocytes B Above: tissue basophil and staff neutrophil. Below: two plasma cells of different sizes, one containing vacuoles Several lymphocytes are also present Marked overproduction of the cells of the so-called "lymphatic tissue" of the bone marrow, with disappearance of the true myelogenous elements

Essential panmyelopathy in an adult. Bone marrow film. Pappenheim staining. From the same case as Figures 191 A., 191 C and 191 D (By courtesy of the University Medical Clinic, Zurich)

Figure 189. Chronic myelogenous leukaemia: basophils, eosinophils, neutrophils and normoblasts in blood (Text on p 14) The large cell to the left of the centre is a neutrophiloblast, above which, slightly more to the left, is an eosinophil with partly basophilic granulation Below the neutrophiloblast, and slightly to the right, are two polychromatic normoblasts while further to the right, above these, are two segmented basophils Also present are a number of staff form and segmented neutrophils Simultaneous "primary" overproduction of various species of blood cells The entrance of erythroblasts into the blood stream is usually a secondary process due either to anaemia or to the "crowding out" of proliferating cells from the bone marrow

Chronic myelogenous leukaemia Blood film. Pappenheim staining

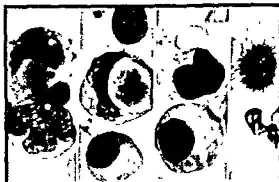
Figure 190. Combined lymphocytic and monocytic leukaemia (Text on p 60) To the left of the centre is a lymphoblast with almost denuded nucleus surrounded by nine denuded lymphocyte nuclei To the left of and above the lymphoblast is a very young monoblast, and in the top right-hand corner are two rather more mature monoblasts In addition, five promonocytes are present. Although the azurophilic granules are scarcely visible, owing to the staining method used, these large cells must be monocytes since they were accompanied by transitional forms to mature monocytes and the peroxidase reaction was negative That they were not early stages of neutrophils, the only other cells with which they could be confused, was evident from the fact that the whole series of neutrophils was present in the same preparation and all stages from the promyelocytes onwards showed the normal strongly positive peroxidase reaction. Three crushed nuclei are also visible

Acute, combined lymphocytic and monocytic leukaemia in an infant. The white blood count fluctuated between 6 100 and 770 000 per cu mm., while the bone marrow contained 65% monocytes and 30% lymphocytes Graham-Knoll peroxidase reaction (By courtesy of the Children's Hospital, Basle, case published by F Hauser [33]).

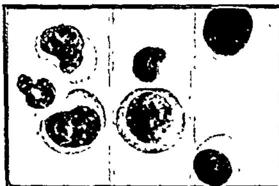
Magnification 1:1200



185 A B C
Lupus erythematosus Phagocytic leucocytes in the bone marrow, so-called "L.E." cells
Phagocytic neutrophils



186. D E F G
D and E phagocytic monocytes, F. disintegrating leucocyte which has not been ingested, G flattened disintegrating nucleus Same case as Fig 185



187. A B C
Lymphatic glandular fever
staff neutrophil, monocytes, plasma cells, typical and atypical lymphocytes in blood



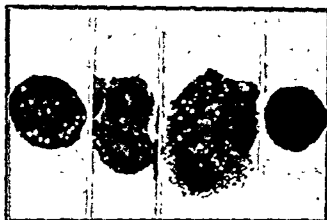
188. A B
Essential panmyelopathy
overproduction of lymphocytes, monocytes, plasma cells and tissue basophils Bone marrow



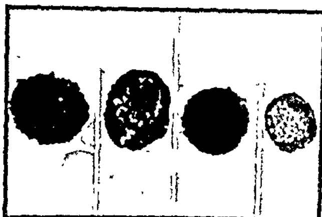
189 Chronic myelogenous leukaemia
basophils, eosinophils neutrophils and normoblasts in blood



190 Combined lymphatic and monocytic leukaemia
Bone marrow



191. A. B C. D
Tissue basophils
A. juvenile form, B mitosis, C flattened and D spherical mature forms Pappenheim staining Bone marrow



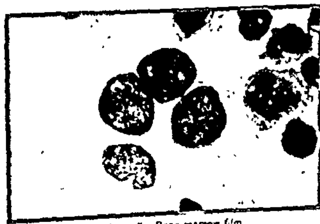
192. E. F. G H
Mature tissue basophils:
E and F. Pappenheim staining G Giemsa staining, H toluidine blue staining Bone marrow



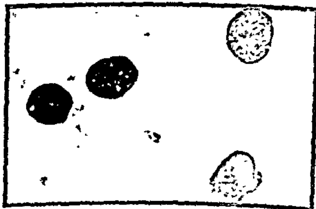
193. Blood vessel with elongated cells in bone marrow film



194. Blood vessel with both elongated and round cells, the latter at the site of rupture Bone marrow film



195. Nuclei of vascular cells Bone marrow film



196. Nuclei of cells of a cutaneous vesse[illegible] [illegible]m

Plate 34

Non-haemopoietic elements in the bone marrow: tissue basophils and vascular cells

Figures 191 and 192. Tissue basophils (Text on p 68) A. Juvenile form with large nucleus of loose structure B Anaphase Very indistinct chromosomes, giving the appearance of a twinning deformity C to H Mature stages C. Well spread out cell containing numerous fine granules D Poorly spread out cell having the appearance of a black sphere and easily mistaken for extraneous material The nucleus is scarcely recognizable and the individual granules cannot be distinguished E Cell with dark, well-stained nucleus F. Cell with insufficiently stained nucleus G Cell with somewhat indistinct granulation Despite Giemsa staining, the granules have not been washed out. H Cell with pale blue nucleus and intense metachromasia following toluidine blue staining

Patients suffering from diseases which gravely inhibit haemopoiesis in the bone marrow. A. C. and D Panmyelopathy, from the same case as Figure 188 B Chronic lymphatic leukaemia, adult. E G and H Acute lymphatic leukaemia, child F Carcinoma with metastases in the bone marrow Bone marrow films A. to F. Pappenheim staining G Giemsa staining H Toluidine blue staining

Figure 193. Bone marrow film containing blood vessel with elongated cells (Text on p 68) Section of a blood vessel, probably of a small artery It is impossible to determine whether the elongated nuclei belong to smooth muscle cells or to endothelial cells.

Adult. Bone marrow film Pappenheim staining

Figure 194. Bone marrow film containing blood vessel with both elongated and round nuclei, the latter at the site of rupture (Text on p 68) Probably from a collapsed venous sinus The cells are most likely to be endothelial cells as their nuclei have assumed a rounded form at the relaxed ruptured end on the right. Vascular cells are mature specialized elements the nuclei of which are rarely recognizable

Adult. Bone marrow film Pappenheim staining

Figure 195. Bone marrow film containing nuclei of vascular cells (Text on p 68) Group of four nuclei from vascular cells Mature specimens without distinct nucleoli Well defined chromatin network. Most of the nuclei have assumed the round relaxed form after being separated from their tissue relationships The lower nucleus is lying in a long characteristically spindle-shaped strip of cytoplasm These cells are probably derived from a venous sinus, because the nuclei of vascular cells from arteries and large veins retain their elongated form after relaxation The uppermost nucleus has a scratch running through it. The change in colour from violet to pure blue is characteristic of mechanical injuries to fixed and stained cells. A neutrophilic myelocyte and a normoblast are also present.

Adult Bone marrow film Pappenheim staining

Groups of round nuclei of vascular cells are common in bone marrow films

Figure 196. Blood film containing nuclei of cells from a cutaneous vessel (Text on p 68) Four naked nuclei of vascular cells, probably from a small vein The nuclei are less spread out than those in Figure 195, but otherwise do not differ from them Here, too, the characteristic arrangement in groups can be seen Similar nuclei were not present elsewhere in the film.

Healthy adult. Film prepared from digital blood obtained after squeezing and rubbing the finger. Giemsa staining

Vascular endothelial cells or their denuded nuclei similar to those shown in Figures 193 to 196 can be found in touch preparations of many organs.

Plate 35

Non-haemopoietic elements of the bone marrow: stroma cells and osteoblasts

In Figures 197, 199 and 200 a magnification of only 1:600 has been used

Figure 197. **Bone marrow film containing poorly spread out stroma cells or fat cells** (Text on p 68) Three stroma cells or their remnants The fat has been dissolved out. In the cell on the left, the thin membranous cytoplasm containing a central nucleus has been preserved whereas in the two cells on the right, it has been largely destroyed

Adult. Bone marrow film. Part of a bone marrow fragment. Pappenheim staining

Figure 198. **Bone marrow film containing a well spread out stroma cell** (Text on p 68) The membranous cytoplasm, which formed a spherical envelope round the fat vacuole, has ruptured, opened out and then become folded over. The border is therefore irregular. The cytoplasm contains a very mature, almost atrophic nucleus without nucleoli, and the fine, oxyphilic reticular structure can still be just distinguished The fat has escaped

Adult. Bone marrow film. Pappenheim staining

Figure 199. **Reticulin networks in stroma cells** (Text on p 68) A. and B The reticulin fibres in the cytoplasm have been made visible by silver impregnation. The fibres are long, uniformly thin and sharply outlined They have stained jet black, in contrast to the violet colour of the other elements. The nuclei are in the centre A. From a thick portion of the film. B From a thin portion of the film.

Child Bone marrow film [34] Gömöri's silver and gold impregnation (p 30)

Figure 200. **Fibrosis of stroma cells in rickets** (Text on p. 68) The thickened, pouch like cytoplasm shows a distinctly fibrous structure even with panoptic staining, the fibres radiating into the surrounding area like a corona The central portion of the cell, containing the nucleus, has been torn out. Finding in "fibrous marrow".

Vitamin-D resistant rickets in a child. Post-mortem bone marrow film Pappenheim staining (By courtesy of the Children's Hospital, Basle)

The stroma or fat cells of the bone marrow do not differ in any way from similar cells in other parts of the body. They are highly specialized cells and do not possess multipotent properties. In the preparation of bone marrow films, the thin membranous cytoplasm of the fragile stroma cells is usually ruptured and the fat escapes.

Figure 201. **Group of osteoblasts** (Text on pp. 68, 69) Four osteoblasts. The nuclei are eccentric and each contains two or three blue nucleoli The cytoplasm is extensive and deep blue with a pale round area of archoplasm at some distance from the nucleus. Below: a monocyte and several lymphocytes and normoblasts

Child with normal bone structure Bone marrow film. Pappenheim staining (By courtesy of the Children's Hospital, Basle)

Figure 202. **Osteoblasts** (Text on pp 68, 69). A. Three small, comparatively young osteoblasts They are thinly spread out and two blue nucleoli are clearly visible in each of the nuclei Areas of archoplasm are also present. B Osteoblast in prophase Coarse sharply outlined chromosomes. The cytoplasm contains pseudovacuoles produced by the incorporation of drops of bone marrow fat. Below lymphocyte

Osteogenesis imperfecta in a child, accompanied by overproduction of osteoblasts Bone marrow film. A. Pappenheim staining, B Giemsa staining (By courtesy of the Children's Hospital, Basle).

Osteoblasts are most likely to be confused with mature plasma cells. The latter, however, are only half the size of osteoblasts, contain no visible nucleoli, and their archoplasm is immediately adjacent to the nucleus.

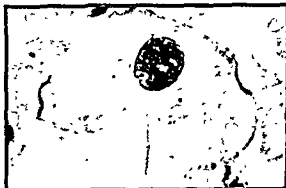
Magnification 1:600 and 1:1200

1:600



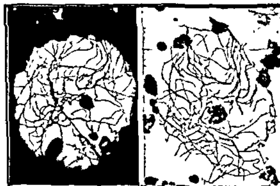
197. Stroma cells (fat cells) of the bone marrow
Pappenheim staining

1:1200



198. Flattened stroma cell of the bone marrow
Pappenheim staining

1:600



199. A B
Reticulin networks. A In a stroma cell in an almost intact fragment of the bone marrow
B in a flattened stroma cell. Gomori staining

1:600



200. Stroma cell fibrosis in rickets. Bone marrow
Pappenheim staining

1:1200



201 Group of osteoblasts. Bone marrow film from a child

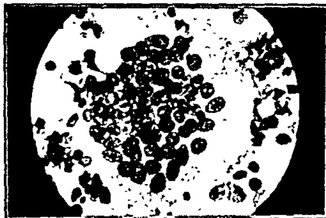
1:1200



202. A B
A. group of osteoblasts, B. osteoblast in prophase
Bone marrow

Magnification 1:450 and 1:1200

1:450



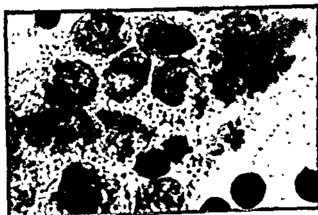
203. Osteoclast. Bone marrow film from a child

1:1200



204. Part of a flattened osteoclast. Bone marrow

1:1200



205. Part of an osteoclast with profuse granulation. Bone marrow.

1:1200



206. Part of an osteoclast containing three diploid nuclei and one pathological, octoploid, giant nucleus which has a boomerang form

1:1200



207. Osteoclastic sarcoma. Osteoclast containing polyploid and hyperdiploid nuclei. Bone marrow

1:1200



208. Osteoclastic sarcoma. Multipolar [illegible] pathological osteoclast. Same case as fig. 207 [illegible]

Plate 36

Non-haemopoietic elements of the bone marrow: osteoclasts

In Figure 203 a magnification of only 1 450 has been used

Figure 203. Osteoclast (Text on p 69). Part of an osteoclast with more than 50 nuclei, each containing a blue nucleolus.

Overproduction of osteoclasts in a child suffering from vitamin-D-resistant rickets Post-mortem bone marrow film Pappenheim staining (By courtesy of the Children's Hospital, Basle).

Figure 204. Part of a thinly spread out osteoclast (Text on p 69) Each of the nuclei contains one and occasionally two distinct blue nucleoli The cytoplasm is blue and contains azurophilic granulation

Child. Bone marrow film Pappenheim staining (By courtesy of the Children's Hospital, Basle).

Figure 205. Part of an osteoclast with profuse granulation (Text on p 69) Thick portion of the film Some of the nuclei are superimposed on others and their nucleoli can hardly be distinguished The cytoplasm contains azurophilic granules, those on the right being particularly coarse, dark violet and gland-like in appearance A few lymphocytes are also present.

Child with normal bone structure Bone marrow film. Pappenheim staining From the same preparation as Figure 201.

Figure 206. Part of an osteoclast containing a deformed nucleus (Text on p 69). Three single diploid nuclei of normal size and one large, boomerang-shaped giant nucleus, probably octoploid Apparently, two of the preceding karyokineses were endomitotic and unaccompanied by division of the nuclei Very rare finding

Child Bone marrow film Pappenheim staining. From the same preparation as Figure 204.

Figure 207. Osteoclast sarcoma (Text on p 69) Pathological osteoclast containing six polyploid and four hypodiploid nuclei

Osteosarcoma of the right femur Touch preparation from the bone marrow Pappenheim staining (By courtesy of Dr P. Lopes Cardozo, Leyden [35])

Figure 208. Osteoclast sarcoma Pathological multipolar mitosis of a polyploid nucleus with slender chromosomes

From the same preparation as Figure 207.

In addition to a few osteoclasts of normal appearance, the preparation also contained definite transitional forms exhibiting pathological heteroploidy, the elements shown here being extreme examples

Only fragments of osteoclasts are found in bone marrow preparations. Osteoclasts differ from megakaryocytes in having numerous unconnected nuclei, of approximately uniform size, each containing a solitary nucleolus Moreover, the granules vary in size and the cells do not agglutinate with blood platelets. The osteoclasts of mammals also resemble in appearance the osteoclasts of lower vertebrates, which have no megakaryocytes

Osteoblasts and osteoclasts are found only in the bones, and occur in particularly large numbers during childhood, when they take part in bone growth. In adults they are usually rare. Osteoblasts were first described and clearly differentiated by M Esser [36]

Plate 37

Non-haemopoietic elements in bone marrow: cells of tumour metastases

Figure 209. Metastasis of a stomach carcinoma in the bone marrow: cancer cells with overproduction of the connective tissue (Text on p. 69). Large specimens detached from a compact mass of tissue. The coarse chromatin network is clearly visible. The nucleus below is in early prophase Most of the cytoplasm has been destroyed, the remainder being indistinct and of a pale, uneven blue colour. Between the cells, strips of pink connective tissue can be seen.

Cancer of the stomach. Bone marrow film. Pappenheim staining. (By courtesy of the University Medical Clinic, Zurich).

Figure 210. Metastasis of a stomach carcinoma in the bone marrow: cancer cells with azurophilic granulation (Text on p 69). Three cancer cells of a different type from those in Figure 209, although the primary tumour is also a stomach cancer They are derived from looser tissue and their cytoplasm has therefore suffered less injury during the preparation of the film. The nuclei are lobed and irregular, and the chromatin network is indistinct. Some large nucleoli may be seen glistening through the chromatin. The cytoplasm is pale blue with azurophilic granulation. No intracellular connective tissue is present.

Cancer of the stomach. A different case from that in Figure 209 Bone marrow film. Pappenheim staining (By courtesy of the University Medical Clinic, Zurich)

As Figures 209 and 210 demonstrate, the cancer cells of metastases in the same organ may differ greatly from one patient to another.

Figure 211. Metastatic cancer cells in the bone marrow (Text on p. 69) A. Above: mitosis of a polyploid cancer cell Short, thick, sharply-defined chromosomes of irregular arrangement. The number of chromosomes is higher than normal, indicating that the cells are polyploid Here, too the pink colour of the intercellular collagen fibres can be clearly seen This preparation contains a number of scratches running diagonally from top to bottom. B Large, mononuclear polyploid cancer cell containing four giant, blue nucleoli; from a bone marrow metastasis To the right: damaged tissue basophil

A. Cancer of the stomach Bone marrow film From the same case as Figure 209 B. Carcinomatosis From the same case as Figure 232. (By courtesy of the University Medical Clinic, Zurich)

Figure 212. Metastasis of a true sympathogonioma in the bone marrow (Text on p 69) The cells are of various sizes and, in most cases, the cytoplasm is ragged or torn off This appearance is characteristic of cells in films prepared from compact tissue and contrasts with the well-defined outlines of the cells in the two following pictures

Patient with a large adrenal tumour, identified histologically as a sympathogonioma Bone marrow film. Pappenheim staining (By courtesy of Dr. A. Piney, London. Case published by Piney, Mallarmé, Ross, Kipfer and Bernard [37]).

Figure 213. "Sympathogonia" in the bone marrow in "sympathogonioma"; perhaps monoblasts in monoblastic leukaemia (Text on p 60) Vacuolated, sharply outlined cells with dark blue cytoplasm Above: cell in metaphase Short, thick chromosomes with indistinct outlines. To the right of this cell is a neutrophilic myelocyte Unlike the cells of tumour metastases or other compact tissues, these cells are not connected with one another but are lying singly side by side like stem cells of blood corpuscles

So-called "sympathogonioma" (neuroblastoma sympathicum) Bone marrow film. Pappenheim staining. From the same case as Figure 214. (By courtesy of the Children's Hospital, Basle).

... the blood in "sympathogonioma"; perhaps monoblasts in monoblastic leukaemia (Text ... like that of the ... pparently mono- ... apparently a pro- ... monocyte, the next stage of development.

So-called "sympathogonioma". Blood film. Pappenheim staining From the same case as Figure 213.

The bone marrow and the blood contain all stages of development, from blast cells through granulated forms up to typical and atypical monocytes These cells are peroxidase negative Their recognition is aided by the fact that the whole neutrophil series is present and that all the stages, from the promyelocyte I onwards, give a positive peroxidase reaction. This form of sympathogonioma seems to be a monoblastoma accompanied by monoblastic leukaemia, a kind of leukaemia which has a parallel in plasmocytoma accompanied by plasma cell leukaemia [38]. The existence of true sympathogonioma, with release of tumour cells into the blood stream, as in Figure 212, is not questioned by placing these cases in a separate category

Magnification 1 1200



209 Metastasis in the bone marrow from a gastric carcinoma. carcinoma cells with production of connective tissue



210. Metastasis in the bone marrow from a gastric carcinoma carcinoma cells with azurophilic granulation



211. A B

Metastatic carcinoma cells in the bone marrow: A. polyploid mitosis, B polyploid, mononuclear cell containing giant nucleoli.



212. Metastasis in the bone marrow from a true sympathogonioma

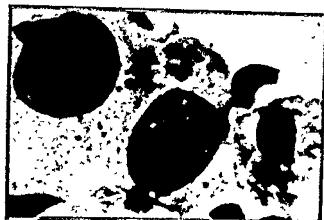


213 "Sympathogonia" in the bone marrow in "sympathogonioma" Monoblasts in monoblastic leukaemia?



214. A B

"Sympathogonia" in the blood in "sympathogonioma Monoblasts in monoblastic leukaemia?



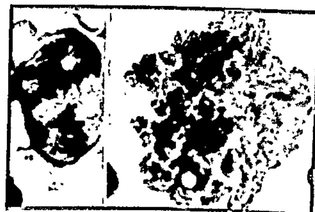
215. Metastasis in the bone marrow from a melanosarcoma
Highly polyploid mononuclear cells containing melanin



216. Metastasis in the bone marrow from a melanosarcoma
Highly polyploid multinuclear tumour cell, which does not contain pigment (so-called leuco form)



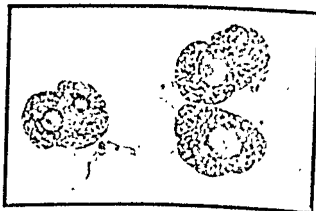
217. Lymphogranuloma Sternberg giant cells
Left, intact cell, right, flattened cell containing typical giant nucleolus Lymph gland punctate



218. A B
Lymphogranuloma - Sternberg giant cells
A. Multipolar mitosis with small chromosomes B endomitosis with large chromosomes Lymph gland punctate



219. Lymphogranuloma: highly polyploid Sternberg giant cell with several highly polyploid nuclei.
Lymph gland punctate



220. A
Lymphogranuloma [illegible] ... [illegible] giant cells containing giant nucleoli Lymph [illegible]

Plate 38

Non-haemopoietic elements of the bone marrow: cells from tumour metastases

Figure 215. Metastasis of a melanosarcoma in the bone marrow (Text on p 69) Two highly polyploid mononuclear monster cells and a fairly mature epithelial cell the cytoplasm of which contains dark masses of melanin

Patient with a melanosarcoma originating from a pigmented naevus Bone marrow film Pappenheim staining (By courtesy of the University Medical Clinic, Zurich).

Figure 216. Metastasis of a melanosarcoma in the bone marrow (Text on p 69) A giant polyploid cell containing 5 nuclei and giant nucleoli Above on the left is a mononuclear tumour cell The cytoplasm has alveolar vacuolation but does not contain any pigment.

Patient with a melanosarcoma originating from a pigmented naevus So-called "leucoform", the majority of the tumour cells being free from pigment. Only a few of the more mature cells contained pigment and these resembled the cell on the right in Figure 215 Bone marrow film Pappenheim staining (By courtesy of Dr B Wiedermann, Olomouc, Czechoslovakia)

Figure 217. Lymphogranuloma · Sternberg giant cells (Text on pp 69, 70) Relatively young mononuclear cells, still only slightly polyploid The intact cell on the left contains several nucleoli but they are rather indistinct The cell on the right is well spread out and the single, blue giant nucleolus can be clearly seen Three lymphocytes are also present.

Lymph gland punctate from a patient with lymphogranuloma Pappenheim staining From the same preparation as Figures 218 and 219. (By courtesy of the University Medical Clinic, Utrecht)

Figure 218. Lymphogranuloma: Sternberg giant cells (Text on pp 69 70) A. Multipolar mitosis with relatively small chromosomes Mitoses of this type give rise to polynuclear lymphogranuloma cells the nuclei exhibiting a low degree of polyploidy B Cell containing one or two highly polyploid giant nuclei in endomitosis, the chromosomes are relatively large Mitoses of this type give rise to lymphogranuloma cells with giant nuclei and often containing giant nucleoli.

From the same preparation as Figures 217 and 219

Figure 219. Lymphogranuloma: highly polyploid Sternberg giant cell with several highly polyploid nuclei (Text on pp 69, 70). No clearly visible nucleoli Above on the right there is also a mononuclear lymphogranuloma cell.

From the same preparation as Figures 217 and 218

Figure 220 Lymphogranuloma: Sternberg giant cells (Text on pp 69, 70) A. and B Three well spread out Sternberg giant cells Loose nuclear structure, typical blue giant nucleoli The cytoplasm, which is blue in A. and pale pink in B, has been largely destroyed

Lymphogranuloma. Lymph gland punctate Pappenheim staining (By courtesy of the University Medical Clinic Lausanne)

The discovery of giant cells with enormous blue nucleoli in the lymph gland punctate, taken in conjunction with the clinical findings, confirms the diagnosis of lymphogranuloma. Large cells with giant nucleoli may, however, also be found in *other diseases and are due to severe mitotic disturbances, cf. Figures 35, 36 211 B*

Plate 39

Non-haemopoietic elements in bone marrow: adventitious epithelial elements

In Figure 225 a magnification of only 1:600 has been used

Figure 221. Cutaneous scale (keratinized epithelium) (Text on p 19). Sharply outlined, wrinkled polyhedral element with no nucleus

Bone marrow film. Pappenheim staining.

Cutaneous scales very often occur as extraneous elements in bone marrow films as well as in blood films. They are generally found singly, but sometimes in groups, and may be covered with bacteria

Figure 222. Nucleated cutaneous epithelium (Text on p 19) Keratinized, epidermal epithelium, with several folds and wrinkles but still containing a nucleus Surrounding it are some some polychromatic erythroblasts and a few unidentifiable cells.

Bone marrow film. Pappenheim staining.

Figure 223. Sebaceous gland cells from the skin (Text on p. 19) A. and B Large, alveolar, peroxidase-negative elements. The sebaceous matter has been dissolved out from the alveoli These should not be confused with fat cells (stroma cells) of the bone marrow

Bone marrow film Graham-Knoll peroxidase reaction.

Figure 224. Sebaceous gland cells in skin film (Text on p 19) A. and B Cells similar to those in Fig 223, but B is a somewhat less mature specimen and contains fewer vacuoles

Skin film prepared from the point of a sternal puncture needle: a piece of skin was removed from the sternal region of the victim of an accident shortly after death and then punctured with the needle Pappenheim staining

This film was prepared in order to ascertain which elements are likely to be transferred by the needle from the skin to the sternal marrow during marrow puncture In addition to sebaceous gland cells, skin films contain cutaneous scales, tissue basophils vascular cells, true fat cells, hair follicles and sweat gland cells Of these elements, only the tissue basophils vascular cells and true fat cells (stroma cells) are also produced in the bone marrow The remainder are elements specific to the skin.

Figure 225. Hair root (Text on p 19) Root of a hair with hair follicle still attached.

Bone marrow film. Giemsa staining

Figure 226. Epithelial cell and bacteria from the mucous membrane of the buccal cavity (Text on p 19) Large typical mucosal cell of the buccal cavity, covered with various kinds of bacteria

Bone marrow film. Pappenheim staining

Epithelial cells from the buccal mucosa may be expelled on to the bone marrow film with drops of saliva when speaking during the preparation of the film. If only isolated epithelial cells are observed, surrounded by bone marrow elements as in the illustration, this indicates that the drop of saliva has fallen into the aspirated marrow before the film was prepared If they are seen lying in a group *surrounded* by a ring of haemolyzed cells, the drop must have fallen on to the dried preparation before fixing Contamination with mucous epithelial cells may, of course, also occur in blood films.

Magnification 1 600 and 1 1200

1 1200



221. Contamination of bone marrow film cutaneous scale (keratinized epithelium)

1 1200



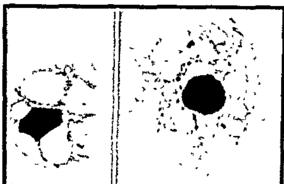
222. Contamination of bone marrow film cutaneous epithelial cell with nucleus

1 1200



223 A B
Contamination of bone marrow film sebaceous gland cells from the skin

1 1200



224. A B
Sebaceous gland cells in skin film

1 600

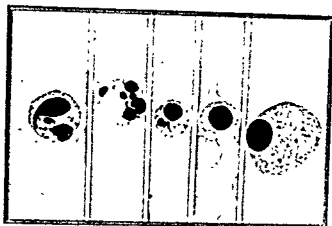


225. Contamination of bone marrow film hair root

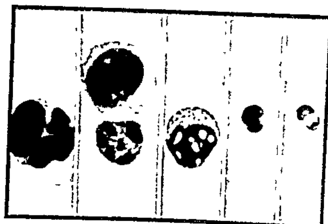
1 1200



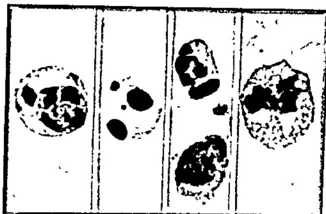
226. Contamination of bone marrow film epithelial cell covered with bacteria from the buccal mucosa



227. A. B C. D E.
Necrobiotic disintegrating forms of leucocytes in blood.



228. F. G. H I K
Necrobiotic disintegrating forms of leucocytes in blood.



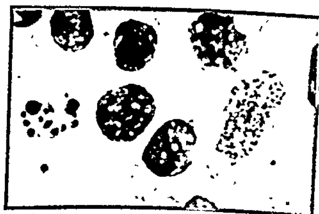
229. A. B C D.
Necrobiotic disintegrating forms of leucocytes
in bone marrow



230. A. B. C D
Artificially produced necrobiotic disintegrating forms
of leucocytes in normal blood



231. A. B C.
A. necrobiotic disintegrating form of a megaloblast.
B polychromatic normoblasts with structureless nuclei.
C. necrotic disintegrating form of a neutrophilblast.



232. Necrobiotic disintegrating forms of metas[illegible] cancer cells
in bone marrow

Plate 40

Disintegrating forms

Figures 227 and 228 Necrobiotic disintegrating forms of leucocytes in the blood (Text on pp 10, 11, 18 70) A. *Necrobiotic cell, probably a monocyte* B C, and D *Necrobiotic forms of neutrophils. In B. and C the liquefied chromatin has broken up into several drops and in D it has condensed to a single large drop.* E. *Necrobiotic form of a promonocyte* F *Part of a megakaryocyte nucleus in the process of destruction The basophilic chromatin is viscous and has broken up into single drops The oxyphilic chromatin is less viscous and holds the nuclear fragment together* G. Below: *necrobiotic form of a promonocyte. The basophilic chromatin has broken up into fragments and is surrounded by the oxyphilic chromatin A narrow border of cytoplasm is just visible around the periphery Above: intact promonocyte.* H *Necrobiotic form of a promonocyte The basophilic chromatin is coherent but contains holes through which the oxyphilic chromatin is visible* I. and K. *Remnants of destroyed leucocytes, no longer capable of identification*

A. C. D and I Healthy individuals B. Haemolytic disease of the new born E. G. and H Monocytic leukaemia with predominance of monoblasts and promonocytes From the same case as Figures 123 and 125 C F. Chronic myelogenous leukaemia. K. Lymphatic glandular fever Blood films A to E., G. and H Pappenheim staining F. I. and K. Giemsa staining

Figure 229 Necrobiotic disintegrating forms of leucocytes in the bone marrow (Text on pp. 10, 11, 18, 70) A. *Necrobiotic form of a monocyte.* B *Necrobiotic form of a leucocyte no longer capable of identification The nucleus has separated into four drops of basophilic chromatin, three of which are surrounded by a narrow border of oxyphilic chromatin* C Above: *necrobiotic form of a neutrophilic promyelocyte II. The basophilic chromatin has become emulsified and is dispersed in the form of drops throughout the oxyphilic chromatin. Both chromatin components are peroxidase negative, as are all nuclear substances On the other hand, the cytoplasm gives a strongly positive peroxidase reaction. This cell demonstrates that the pink substance surrounding the drops of basophilic chromatin in necrobiotic cells is oxyphilic chromatin and not cytoplasm* Below: *intact peroxidase-positive promyelocyte II.* D *Necrobiotic form of a leucocyte no longer capable of identification. Probably necrobiosis following an arrested mitosis, as can be deduced from the rosette shape of the nucleus and the mottled appearance of the cytoplasm*

A. Agranulocytosis B. C. D. Leukaemias A. and C. Graham-Knoll peroxidase reaction, B Giemsa staining D Pappenheim staining.

Resting nuclei or chromosomes containing homogeneous dark lumps, as in D, do not always belong to true necrobiotic cells They may also result if the films are fixed too soon after preparation, if fixing solutions containing water are used, or if the films accidentally come into contact with alkali or acid The lumps may be situated in the centre of the nucleus or they may form a broad ring around the circumference As a result of this separation, the remainder of the nucleus is poor in chromatin and therefore stains weakly The chromatin structure is lost Such artificial products can be easily distinguished from true necrobiotic cells because all the nucleated elements, at least in the affected part of the film exhibit a similar appearance If other preparations are made from the same case and are fixed and stained carefully, perfectly satisfactory staining takes place, indicating the artificial nature of the changes

Figure 230. Necrobiotic disintegrating forms of leucocytes produced artificially in vitro in normal blood (Text on p 70). A. B and D *Necrobiotic forms of neutrophils* C *Necrobiotic forms of two neutrophils, two eosinophils and one monocyte (in the centre) The nuclei are liquefied and therefore structureless, and have either broken up into small droplets or condensed to form a single drop The neutrophil in A (lower cell) resembles a normoblast in appearance This is not the case however since the individual from whom the blood was taken was in good health and had no normoblasts in the blood Moreover a peroxidase-positive neutrophil with a similar necrobiotic nucleus can be seen in D; a normoblast would be peroxidase negative in histological sections necrobiotic forms of eosinophils with round, condensed nuclei, like the lower cells in C, are liable to be mistaken for eosinophilic myelocytes*

Three healthy individuals Films from the leucocyte layer of a sample of citrated blood which had stood at room temperature for 48 hours A. B and C Giemsa staining D. Graham-Knoll peroxidase reaction

Figure 231. Necrobiotic forms of erythroblasts and necrotic form of a neutrophiloblast (Text on pp 10, 11, 18, 70). A. *Necrobiotic disintegrating form of a basophilic megaloblast, the development of which has been arrested,* cf Figure 33 C B *Two polychromatic normoblasts with structureless (necrobiotic) nuclei In the upper normoblast, the nucleus has been partially forced out mechanically like that of the monocyte in Figure 230 C Normoblasts with necrobiotic nuclei are the normal precursors of normocytes* C *Necrotic disintegrating form of a neutrophiloblast. That the cell is necrotic and not necrobiotic is shown by the fact that the nucleus no longer contains basophilic chromatin but is completely decolorized The cell is surrounded by intact neutrophiloblasts, normoblasts and a Gumprecht's shadow*

A. Untreated pernicious anaemia B Anaemia in a child C. Neutrophiloblastic leukaemia From the same case as Figure 100 Bone marrow films A. Giemsa staining B Graham-Knoll peroxidase reaction. C Pappenheim staining

Figure 232. Necrobiotic forms of metastatic cancer cells in the bone marrow (Text on p 70) *Partly intact, partly disintegrating tumour cells from a necrotic bone marrow metastasis*

Carcinomatosis Bone marrow film Pappenheim staining From the same case as Figure 211 B (39).

Plate 41

Artefacts

Figures 233 to 238. Various kinds of artefacts produced from blood corpuscles and bone marrow cells (Text on pp 70, 71)

A. Stricture formation in a normal mononuclear lymphocyte The cell is constricted in the middle. The normocytes lying near the constriction have also been damaged If the cell had been completely divided, two artificial microlymphocytes would have been produced Artefacts of this kind are rare, but may be produced from any species of blood corpuscle

B Below: crushed segmented neutrophil Above: intact neutrophil When a segmented leucocyte is crushed, the number of chromatin patches produced is generally equal to the number of nuclear segments present in the intact cell The "postnucleolar chromatin masses" (lower middle segment) are clearly visible

C. Crushed monocyte. When a monocyte is crushed, the nucleus shrinks, becomes very small and adopts a boomerang shape The chromatin is therefore more compact than that of crushed juvenile neutrophils, which are otherwise similar in appearance

D. Crushed lymphocyte The blue nucleolus which, in the intact mature lymphocyte, is always hidden by the dense covering of chromatin, now becomes clearly visible Most of the cytoplasm has been detached and has partly dissolved in the surrounding blood plasma, the remainder being visible below in the form of a small blue droplet ("pseudo blue blood platelet"), see Figure 47 G.

E. Centre: similar fragments of cytoplasm from lymphocytes Above and below: two lymphocytes, which are only slightly deformed.

F. Two crushed lymphocyte nuclei; the cytoplasm has disappeared, leaving the so-called Gumprecht's shadows or nuclear shadows The spaces are not nucleoli. Also present are two intact lymphocytes

G Two intact neutrophilic promyelocytes II for comparison with H Several blue nucleoli, abundant azurophilic granulation

H. Similar cell but from a thin portion of the film and therefore crushed. The chromatin network and nucleoli can be seen more clearly than in the intact cells, and the cytoplasm has been extruded forming long projections

I Above: another crushed neutrophilic promyelocyte II, giving a positive peroxidase reaction. Below: intact promyelocyte II. The cells H and I. above are known as "Ferrata cells".

K. Neutrophilic promyelocyte II with nuclear projections into the cytoplasm Such nuclear projections are easily produced when blood is mixed with citrate solution

L. Above: megaloblast. One half of this cell has been pressed flat (Ferrata stage), while the other half has been crushed (Gumprecht's shadow) Below: megaloblast with cytoplasmic projections

M Flattened nucleus of a stroma cell (fat cell) of the bone marrow; the remains of the cytoplasm have been drawn out in a spindle shape Below to the left is a normoblast.

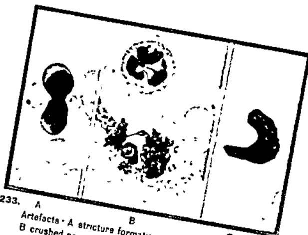
N Below: tetraploid lymphocyte with two diploid nuclei The nuclei have been forced apart and the cytoplasm has contracted in the centre (stricture formation) Above: normal lymphocyte.

O Octoploid basophilic normoblast with four diploid nuclei The bottom nucleus has been partly expelled and a constriction has formed in the cytoplasm between it and the other nuclei. Also present are a normoblast with rosette-shaped nucleus, and a nuclear shadow

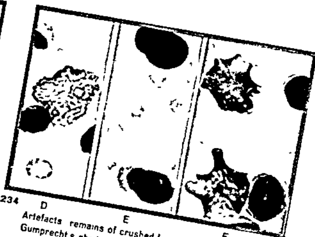
P. Octoploid basophilic normoblast with four diploid nuclei A constriction has formed in the centre, as a result of the forcing apart of the two halves, each of which has two nuclei Above: three normoblasts Below: Gumprecht's shadow

It is much more probable that the strictures in M N and O. are mechanically produced, like the one in A., than that they are due to incompleted amitoses.

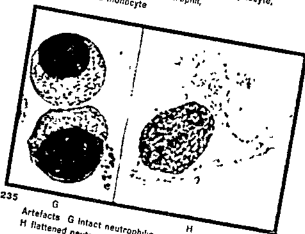
Films prepared both from normal people and from patients A B C. and D blood films, E. and F lymph gland films G to P bone marrow films. K. from bone marrow which was aspirated into citrate solution. A. B. E. F G. H and K. Pappenheim staining, C. D. L. N O and P. Giemsa staining. L and M Graham-Knoll peroxidase reaction.



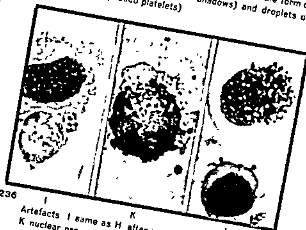
233. A B C
Artefacts · A stricture formation in a lymphocyte, B crushed segmented neutrophil, C crushed monocyte



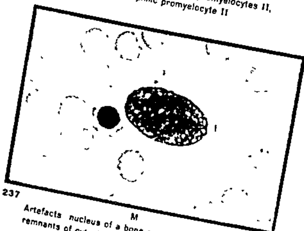
234 D E F
Artefacts remains of crushed lymphocytes in the form of Gumprecht's shadows (nuclear shadows) and droplets of cytoplasm (pseudo platelets)



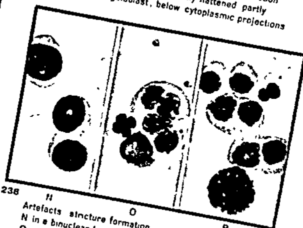
235 G H
Artefacts G intact neutrophilic promyelocytes II, H flattened neutrophilic promyelocyte II



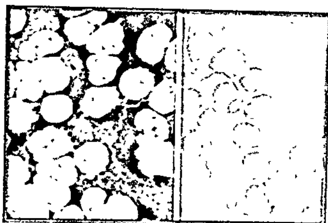
236 I K L
Artefacts I same as H after peroxidase reaction K nuclear projections L partly flattened partly crushed megaloblast, below cytoplasmic projections



237 M
Artefacts nucleus of a bone marrow stroma cell with remnants of cytoplasm

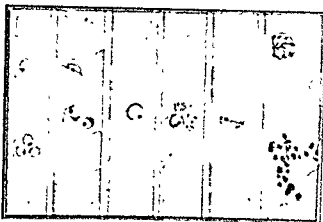


238 N O P
Artefacts stricture formation
N in a binuclear lymphocyte
O and P in two tetranuclear normoblasts



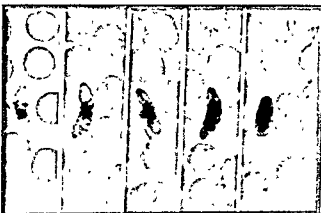
239. A B

Relapsing fever
Spirochaetae recurrentis in blood film.
A. Indian ink preparation, B Pappenheim staining



240 A. B C. D E F.

[illegible] fer [illegible]



241. A B C D E.

Estivo-autumnal malaria Sexual cycle of plasmodium falciparum in blood: A. B C. female gametocytes, D E. male gametocytes Blood films



242. A. B C

Estivo-autumnal malaria A ring forms, B segmenter, C gametocytes Thick drop preparation



243 A. B C D E

Quartan malaria Asexual cycle of plasmodium malariae in blood A. to D band forms E. segmenter Blood films.



244. A B C D

Quartan malaria A B sexual cycle [illegible]
gametocytes Blood films
C rings and D segmenter in th[illegible] [illegible]

Plate 42

Blood Parasites: Spirochaetes, Plasmodium falciparum, Plasmodium malariae

Figure 239. Relapsing fever. Spirochaetes recurrentis in blood film (Text on p 72) **A.** Indian ink film. The spirochaetes c be seen as white, corkscrew-shaped bodies among the round white patches which are the blood corpuscles In the panoptically stain film B., the spirochaetes have stained reddish-violet.

Figure 240. Estivo-autumnal malaria. Asexual cycle of Plasmodium falciparum in blood (Text on pp 72, 73). **A.** and **B** Young ring forms. **C.**, **D** and **E.** Older ring forms. **F.** Segmenter Blood films

The younger ring forms are small in size, especially the three in A. which have invaded a single normocyte In B two parasites have invaded one normocyte The erythrocyte in D contains particles of a dirty violet colour, the so-called **Maurer's dots** *In the segmenter In F. above, the merozoites can be clearly distinguished. The structure in the lower part of F. is a mass of agglutinated blood platelets, and should not be mistaken for parasites.*

Figure 241. Estivo-autumnal malaria Sexual cycle of Plasmodium falciparum in blood (Text on pp 72, 73). **A. B** and **C** female gametocytes **D** and **E.** male gametocytes Blood films

The sexual forms are elongated and some are half-moon shaped. The female gametocyte has blue cytoplasm and a small nucleus surrounded by pigment. the cytoplasm of the male gametocyte is more reddish and it has a larger nucleus and more pigment.

Figure 242. Estivo-autumnal malaria. Thick drop preparation (Text on pp 72, 73) **A.** Ring forms. **B** segmenter. **C** gametocytes

In thick drop preparations, only the stromata of the erythrocytes are preserved, owing to haemolysis The nuclei of the schizonts in A and B are particularly easy to recognize, but the circular form of the cytoplasm has not always been preserved The gametocytes in C are half-moon shaped, but this is not invariably the case in thick drop preparations, as they may sometimes be round Notice the marked shrinkage of the gametocytes in the thick drop preparation as compared with Figure 241 (same magnification)

Figure 243. Quartan malaria. Asexual cycle of Plasmodium malariae in blood (Text on p 73). **A.** to **D** band forms. **E.** segmenter. Blood films

The lower parasite in A is a very young band form the remaining band forms in A. to D being older and more mature, with reddish-violet nuclei, blue cytoplasm and brown pigment. The segmenter in E. contains nine nuclei the pigment being in the centre The size of the invaded erythrocytes is not increased

Figure 244. Quartan malaria. Sexual cycle of Plasmodium malariae in blood and parasites in thick drop preparations (Text on p 73). **A.** and **B** Gametocytes Blood films **C** Ring forms and **D** segmenter in thick drop preparations

The size of the invaded erythrocytes is not increased In D one merozoite is lying a little to the right, away from the main group. The pigment from the parasites has collected at the upper right hand corner of the merozoite group

Figures 240 to 244 B are from preparations supplied by courtesy of the Swiss Tropical Institute Basle

Plate 43

Blood Parasites: Plasmodium vivax, Trypanosomes, Leishmanias, Microfilarias

In Figure 250 a magnification of only 1 : 400 has been used

Figure 245. Tertian malaria. Asexual cycle of Plasmodium vivax *in blood* (Text on p. 73). **A. B and C** Ring forms and amoeboid forms D. and E. Segmenters. F. Free merozoites Blood films.

A. below and B.: young schizonts, ring forms The normocyte in B has been *invaded by two parasites* A above and C.: more mature schizonts, amoeboid forms All the invaded normocytes are markedly *increased in size and have* lost their colour In C, the invaded normocyte contains Schüffner's dots. These are bright red and dispersed *throughout the entire cell* The segmenter in E. contains numerous merozoites. In F., the pigment has collected in the centre of the merozoite group

***Figure 246. Tertian* malaria. Sexual cycle of Plasmodium vivax in blood** (Text on p 73) **A B and C.** Female gametocytes, D and E. male gametocytes Blood films.

The young gametocyte in A. differs from young schizonts in having a compact structure without vacuoles *The female* gametocytes in B and C contain smaller and more compact nuclei than the male gametocyte in D. The nucleus of the *gametocyte in E.* has broken up into small pieces This is the first stage of gamete formation (exflagellation) and is rarely found *in human blood* The normocyte in A. contains pale blue granules, the usual **basophilic stippling** Infected normocytes with basophilic stippling *contain, as in this* preparation, only young parasites The normocyte in B. contains **Schüffner's dots** In B. to E. the normocytes have *been invaded by older* parasites and are increased in size

Figure 247. Tertian malaria. Thick drop preparation (Text on p 73) **A.** Ring forms. **B** Gametocytes.

Numerous ring forms, most of which are greatly deformed. The Schüffner's granules of the *gametocytes are* not very distinct in thick drop preparations.

Figure 248. Trypanosomiasis (Text on p 73) **A.** Trypanosoma gambiense in human blood **B** Trypanosoma *gambiense in* guinea pig blood C. Trypanosoma cruzi in guinea pig blood Blood films

The flagellum, undulating membrane and kinetoplast can be clearly seen, especially in B The kinetoplast and *flagellum are* double because the parasites have begun to divide. The parasite in C., near the top, has a particularly long flagellum, a *characteristic feature* of Trypanosoma cruzi.

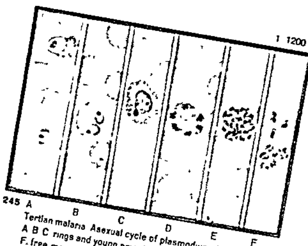
Figure 249. Leishmaniasis (Text on p 74). **A.** and **B** Leishmania donovani in monocytes from human bone marrow *The* monocyte in A. is a well preserved specimen and has a small, atrophic, vacuolated nucleus The cytoplasm contains numerous *parasites* The monocyte in B is very flattened and has a round, atrophic nucleus The parasites can be clearly seen, most of *them are* oval and contain two deeply stained bodies The larger body is the principal nucleus (trophonucleus) the smaller one the *kinetoplast.* Also present in B are a lymphocyte and a normoblast.

In man, leishmanias occur only intracellularly in monocytes

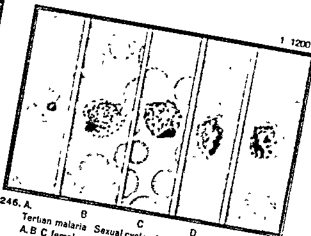
Figure 250. Filariasis (Text on p 74) Left microfilaria (larva) of Loa loa The sheath is clearly visible *and the nuclei* extend as *far as* the end of the tail on the right. Right microfilaria of Dipetalonema perstans It is shorter and thinner *and is* not surrounded by a sheath. The nuclei again extend as far as the end of the tail A few leucocytes are also present.

Mixed infection with Loa loa and Dipetalonema perstans. Thick drop preparation *The* magnification here is only 1 400

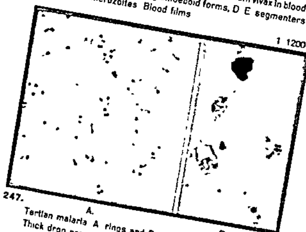
Figures 245 246 247 B, 248 and 250 are from preparations provided by courtesy of the *Swiss Tropical* Institute Basle Figure 249 is by courtesy of Prof. C. Jiménez-Diaz, Dr G *Paniagua* and Dr *J Perianes, Madrid*



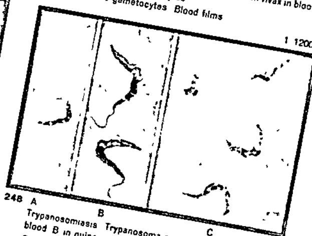
245 A B C D E F
Tertian malaria Asexual cycle of plasmodium vivax in blood. A B C rings and young amoeboid forms, D E segmenters F. free merozoites Blood films



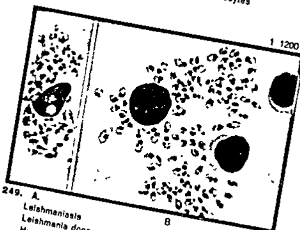
246. A. B C D E.
Tertian malaria Sexual cycle of plasmodium vivax in blood A. B C female gametocytes D E. male gametocytes Blood films



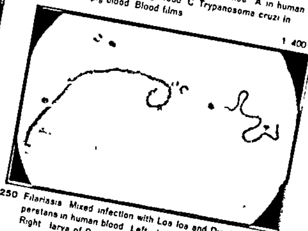
247. A. B
Tertian malaria A rings and B gametocytes Thick drop preparation



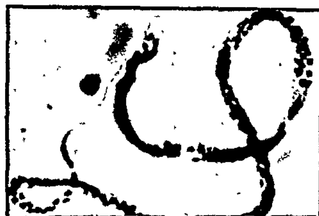
248 A B C
Trypanosomiasis Trypanosoma gambiense A in human blood B in guinea pig blood C Trypanosoma cruzi in guinea pig blood Blood films



249. A. B
Leishmaniasis
Leishmania donovani in monocytes
Human bone marrow film



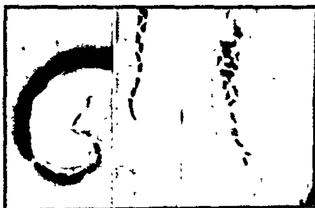
250 Filariasis Mixed infection with Loa loa and Dipetalonema perstans in human blood Left larva of Loa loa Right larva of Dipetalonema perstans



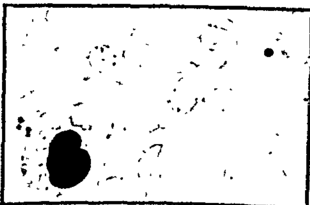
251. Filariasis
Microfilaria bancrofti Blood



252. Filariasis
Microfilaria malayi Blood



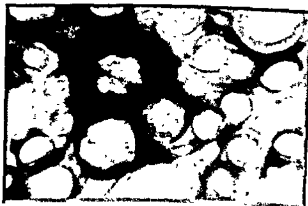
253. A. B C.
Filariasis Ends of tails of microfilariae
A. Loa loa B bancrofti C malayi Blood



254. Carrión's disease Oroya fever.
Bartonella bacilliformis in erythrocytes and in a monocyte
Blood



255. Blastomycosis brasiliensis.
Paracoccidioides in a giant cell Lymph gland puncture.



256 Blastomycosis brasiliensis
Paracoccidioides Lymph gland puncture

Plate 44

Blood parasites: Microfilarias, Bartonella bacilliformis, Blastomyces brasiliensis

Figure 251. Filariasis; Microfilaria bancrofti (Text on p 74) Since Pa[illegible]ed, the sheath does not show up very well The tip of the gradually tapering tail [illegible]
Patient suffering from Filar[illegible] Pappenheim staining (By courtesy of Dr P Lopes Cardozo, Leyden)

Figure 252. Filariasis; Microfilaria malayi (Text on p 74) The long wide sheath is clearly visible In the small circle on the right formed by the microfilaria, the head with the protruding sheath is visible at the bottom, while the point of the tail is at the top At the extreme tip of the tail are two body-cell nuclei, lying to one side and isolated from the other nuclei, a characteristic feature of this species of microfilaria
Patient from the northern shores of Celebes suffering from Filariasis malayi Thick drop preparation Haemotoxylin staining (By courtesy of Prof. C. D de Langen, Utrecht).

Figure 253. Filariasis; tail tips of microfilariae (Text on p 74) A. Loa loa, B. Wucher[illegible] of Loa loa tapers rapidly and the nuclei reach right to the tip Th[illegible] present at the tip The tail of Microfilaria malayi has a blunt [illegible] site and is here bent upwards The tip of the tail contains tw[illegible] to one side of the others
A Thick drop preparation Carazzi staining (By courtesy of Dr. A. Perret-Gentil Basle) B From the same preparation as Figure 251
C. From the same preparation as Figure 252.

Figure 254 Carrión's disease, Oroya fever stage (Text on p 75) Spherical and rod-shaped bartonellas (Bartonella bacilliformis) in erythrocytes and in the monocyte on the left. Above the monocyte are four blood platelets The erythrocyte in the top right hand corner contains a Howell-Jolly body.
Blood film from a patient suffering from Carrión's disease at the Oroya fever stage Pappenheim staining (By courtesy of Prof C. Monge M and Prof P Weiss, Lima)

Figure 255. South American blastomycosis (Text on p 75) Polynuclear giant cell, similar to a Langhans' giant cell, containing a single parasite, Paracoccidioides (Blastomyces) brasiliensis, situated in the top right hand corner The parasites are chromophobic, doubly refracting and have the appearance of small glass spheres in the tissues
Lymph gland punctate from a patient suffering from Blastomycosis brasiliensis Leishman staining (By courtesy of Dr M Pio da Silva São Paulo, Brazil [40]).

Figure 256. South American blastomycosis (Text on p 75) Necrotic lymph gland tissue containing a colony of Paracoccidioides (Blastomyces) brasiliensis The parasites are of various sizes
Lymph gland punctate from the same case as Figure 255 Leishman staining

Literature

The literature on haematology has grown to such an extent that even a list of the more important publications would be beyond the scope of an atlas of this type. For additional information, the reader is referred to the textbooks and manuals of haematology listed below, the majority of which also contain fairly complete bibliographies.—A list of references to publications dealing with cases illustrated in the plates will be found on the following page.

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B. REFERENCES TO PUBLICATIONS ON CASES ILLUSTRATED

1) Fig. 12 B — Schwartz, S. O., Motto, S. A. (1949) Diagnostic significance of "burr" red blood cells. *Amer. J. med. Sci.* **218**, 563—566

2) Figs. 12 B, C — Feulgen, R., Rossenbeck, H. (1924). Mikroskopisch-chemischer Nachweis einer Nuklein- [illegible]

3) Figs. 21 B, C, 174 — [illegible] Entstehung der geformten Gerinnungselemente und der Entkernung der Erythroblasten. *Helv. med acta*, Series A, **13**, 595—624 (Fig. 21 B, 21 C: 613—614, Fig. 174: 599—607).

4) Figs 34—36 E — Owren, P. A. (1948). Congenital hemolytic jaundice. The pathogenesis of the "hemolytic crisis". *Blood* **3**, 231—248

5) Fig 36 F — Goldeck, H (1950) Die Unspezifität der [illegible] *Aerztl* [illegible]

6) Fig. 45 A — [illegible] a leu- [illegible] —385.

7) Figs 55, 59 — [illegible] La leucémie à basocytes, *Sang*, **19**, 193—218.

8) Figs 64 B, 78 A, 80 — [illegible] 254).

9) Figs 64 D, 65, 69 C — Landolt, R (1944). Eosinophiles Leukämoid und Lymphogranulomatose *Schweiz. med. Wschr.* **74**, 1071—1075.

10) Figs 67, 68 — Piney, A (1949) La leucémie à éosinophiles existe-t-elle? *Rev. Hématol* **4**, 3—5.

11) Fig 77 A — Haverkamp Begemann, N., van Lookeren Campagne, A (1951) Homozygote vorm der kernanomalie van Pelger-Huet. *Maandschr. Kindergeneesk* **19**, 338—342.
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